SELECTIVE DECONTAMINATION OF THE DIGESTIVE TRACT

A method for infection prevention in granulocytopenic patients

H. G. DE VRIES-HOSPERS

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STELLINGEN

I.

Bij patienten met minder dan $0{,}5 \times 10^9$ granulocyten per l bloed is selectieve decontaminatie aangewezen ter preventie van infectie.

II.

Het toepassen van selectieve decontaminatie zonder „omgekeerde isolatie" van de patiënt is verantwoord, doch vereist bacteriologische bewaking.

III.

Er dient onderzocht te worden of selectieve decontaminatie het optreden van virale infecties beïnvloedt.

IV.

Bij patiënten die langdurig beademd worden, verdient het aanbeveling om in geval van een infectie zoveel mogelijk gebruik te maken van middelen die geen „overgroei" met resistente potentieel pathogene microörganismen in de oropharynx tot gevolg hebben.

V.

De huidige hausse in de publiciteit rond interferon valt te betreuren, aangezien de bij het publiek gewekte verwachtingen waarschijnlijk niet waar gemaakt kunnen worden.

VI.

Veel Coxsackie B virusinfecties zullen niet kunnen worden bewezen, wanneer wordt uitgegaan van het standpunt, dat het virus eerst uit de faeces geïsoleerd dient te worden en pas in tweede instantie serologisch onderzoek met betrekking tot het geïsoleerde virus kan worden gedaan.

VII.

Bij het zoeken naar de oorzaak van koorts na orgaantransplantatie, zal het virologisch laboratorium een grotere rol moeten spelen door het snel aantonen van viremie of antigenemie met betrekking tot virussen uit de herpesgroep.

VIII.

Bij cytostatische behandeling wegens gemetastaseerd mammacarcinoom is de aanwezigheid van levermetastasen niet van prognostische betekenis.

IX.

Kort-durende infusies met ara-C resulteren niet in een effectieve cytostatische spiegel in de liquor.

X.

Na bestraling van het halsgebied, bijvoorbeeld wegens de ziekte van Hodgkin, is regelmatige klinische en biochemische controle van de schildklier vereist.

XI.

Of correctie van de zuurgraad noodzakelijk is bij een ketoacidotisch coma, hangt voornamelijk af van de cardiale toestand van de patiënt.

XII.

De stelling dat de behandeling van het panaritium dient te geschieden door de chirurg, klopt als een zwerende vinger.

Stellingen
behorende bij het proefschrift van
H. G. de Vries-Hospers

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RIJKSUNIVERSITEIT TE GRONINGEN

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PROEFSCHRIFT

ter verkrijging van het doctoraat in de geneeskunde
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op gezag van de Rector Magnificus Dr. M. R. van Gils
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geboren te Gieterveen

1981
DRUKKERIJ VAN DENDEREN B.V.
GRONINGEN

Promotor:	Prof. Dr. D. van der Waaij
	Prof. Dr. H. O. Nieweg
Co-promotor:	Prof. Dr. J. B. Wilterdink
Referent:	Dr. N. H. Mulder

This study has been performed in the:
Laboratory for Medical Microbiology and the
Department of Clinical Bacteriology – Regional Laboratory of Public Health
in collaboration with the
Haematological Division of the Department of Internal Medicine
University Hospital of Groningen.

Aan mijn ouders
Aan Jaap

Een ieder, die heeft meegedacht, meegewerkt, meegeleefd of op welke wijze dan ook belangstelling heeft getoond, wil ik hartelijk bedanken.

CONTENTS

CHAPTER I

INTRODUCTION

Treatment with oncostatic and/or immunosuppressive agents makes the patient extremely susceptible to infections (1, 2, 3, 4). An increased susceptibility is also observed in bone marrow aplasia of other origin (5, 6). Most of the episodes occur during periods of severe neutropenia. In 1966 Bodey described the relation between the peripheral leukocyte count and the infection risk (7). The incidence of infection was found more closely related to granulocytopenia than to lymphopenia. Both the degree and duration of the granulocytopenia appeared to influence the chance that an infection develops. This risk approaches 100% if granulocyte levels below 100 cells/mm^3 of blood persist for more than 3 weeks. Patients with haematological malignancies, especially those with acute leukaemia, often experience neutropenia for prolonged periods of time, either due to the disease or to its treatment. Infectious complications, predominantly caused by aerobic gram-negative rods (2, 3, 8, 9), yeasts and fungi (10, 11, 12, 13), contribute to the high mortality rate in these patients (8, 10), even when adequate antimicrobial therapy is started at the first signs and symptoms of infection.

During a ten year study period, which lasted from 1954 to 1963, 70% of the patients with acute leukaemia died as a result of infection (1). While the incidence of fatal staphylococcal infections decreased during this period, an increase in fungal infections and *Pseudomonas* septicaemia was noted. In the series of Burgess et al. (2) septicaemia was considered to be the major cause of death in 61.2% of the patients. In 31 out of 71 episodes of septicaemia (43.6%) *Escherichia coli* and *Pseudomonas aeruginosa* were involved. In a study recorded by Schimpff (14) 70% of the death in patients with acute leukaemia was caused by infection. He also found that the majority of the bacteriologically documented infections were caused by the patient's own gastro-intestinal microflora. However, 47% of these bacteria were found to be acquired in the hospital since they were not detected in the cultures on admission. It might be assumed therefore that treatment of the patient in effective isolation rooms should result in a predictable reduction of infections caused by the hospital-acquired microorganisms. An even greater decrease of the infections was to be expected from antibiotic decontamination of the resident microflora.

During the last decade many studies have been conducted on this subject. Protective isolation alone appeared to result in only a slight reduction of the infection incidence (15), not nearly reaching the equivalent of the 47% of the cases in which hospital-acquired bacteria had been found by Schimpff. The effect of oral non-absorbable antibiotics without protective isolation did result in a reduction of the number of infections in one report (16), but was not effective in another study (17). In some cases severe infections have been noticed in this situation (18, 19).

A more definite effect in infection prevention was obtained with the combination of isolation and gut sterilization (16, 17, 20, 21, 22). The results of these combined prophylactic measures varied somewhat depending on the kind of patients treated and showed improvement in the course of the years.

However, infection prophylaxis by gut sterilization in a protected environment can only be performed in hospitals with a highly specialized team and expensive facilities for appropriate isolation. What could be done in hospitals that lack this equipment for reverse isolation? One could be tempted to just sterilize the gut with oral non-absorbable antibiotics. However, this measure could be expected to have a thoroughly undesirable effect: overgrowth with resistant microorganisms.

This is a life threatening risk for granulocytopenic patients. Total decontamination without isolation must therefore be considered potentially dangerous. The mechanism of the above mentioned complication has been partly elucidated in the last years.

Gut sterilization with as purpose to eradicate all microorganisms, includes the elimination of the anaerobic flora of the alimentary canal. These anaerobes have an important "colonizing inhibiting" function which has been called Colonization Resistance (CR). The mechanism of the CR has been described first by Van der Waaij (23). In animal studies it was shown that oral contamination of germ-free animals with bacteria resulted in a high concentration of these bacteria in their entire digestive tract and in their faeces. To obtain similar results in conventional animals for a short time, extremely high oral doses of bacteria were necessary.

This colonization preventing effect appeared to be the result of the concerted action of host and anaerobes. Gut sterilization, aimed at elimination of all bacteria, therefore results in a considerable decrease of the CR of the digestive tract. This makes in such totally decontaminated patients reverse isolation of the patient inevitable. Isolation is necessary to substi-

tute for the normal endogenous barrier of microbial origin (i.e. the CR) which is destroyed by antibiotics. This is even more important when the environment harbours many resistant bacteria, e.g. in a hospital.
In neutropenic patients there is little reason to eliminate the anaerobes, since they rarely cause infections in the compromised host (1, 9). On the contrary, most of the infections are caused by aerobic gram-negative rods and yeasts. The majority of these aerobic bacteria can – as mentioned above – be found in the oropharyngeal- or gut flora prior to infection.
The observation that most infections are caused by aerobic bacteria from the endogenous gastro-intestinal microflora, as well as the beneficial role of anaerobes in the CR, prompted us to search for methods to more or less selectively eliminate the potentially pathogenic aerobic bacteria from the digestive tract of patients, without affecting the favourable influence of the anaerobes. For this purpose antibiotics were selected on the basis of their "selective decontaminating" capacity in animals (24, 25, 26).
As far as aerobic gram-negative rods are concerned, nalidixic acid, co-trimoxazole and polymyxin appeared suitable. The first two drugs are absorbed from the gut after oral administration. Therefore, relatively high dosages are required to accomplish a static or even cidal concentration in the gut lumen as to achieve decontamination. For suppression of yeasts and fungi, amphotericin B and nystatin are mostly used. Since these drugs do not affect bacteria, they are both considered suitable for selective decontamination.
The non-absorbable polymyxin, amphotericin B and nystatin meet the criteria for gastro-intestinal decontamination as proposed by Poth (27) much better than the absorbable drugs.
The present thesis deals with a clinical and microbiological evaluation of this method of selective decontamination of the digestive tract (SDD) for prevention of infections.
In Chapter II the preliminary results concerning clinical data of a prospectively controlled randomized trial on the use of the drugs for SDD in adequate dosages on the prevention of infection in granulocytopenic patients are reported. The effect of SDD on the incidence of infections was compared with quantitative and qualitative data with regard to the load of aerobic potentially pathogenic bacteria in the digestive tract of the patients (Chapter III).
The question immediately connected with the oral use of antimicrobials, namely the question about the emergence of resistant strains has been dealt

with in Chapter IV. Special attention has been paid to the efficiency of selective decontamination of potentially pathogenic yeasts. The majority of the erosions and infections caused by these microorganisms are localized in the oropharynx and the esophagus.

Candida-associated lesions or ulcers usually present as milk-white areas on the mucosa of the oropharynx. From these lesions the yeasts may grow invasive and cause septicaemia or deep systemic mycoses. Mycotic infections are difficult to cure and have a high mortality rate.

Since most of the *Candida*-associated lesions first appear in the oropharynx, we have searched for methods to establish and maintain high concentrations of an antimycotic drug in the saliva. One study involved the application of the drug in the mouth on the gums in orabase® (Chapter V). This is a methylcellulose paste which strongly adheres to moist surfaces like the mucous membranes. Different drugs including amphotericin B can be mixed with this paste and are gradually released after application. In Chapter VI a study of the result of application of amphotericin B as a lozenge on the salivary concentrations is described. The effect of amphotericin B lozenges on the suppression of oral *Candida* proliferation and colonization in severely granulocytopenic patiens with acute leukaemia was subsequently studied (Chapter VII).

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CHAPTER II

PROSPECTIVELY RANDOMIZED TRIAL ON THE EFFICACY OF SELECTIVE BOWEL DECONTAMINATION IN INFECTION PREVENTION IN THE COMPROMISED HOST. PRELIMINARY RESULTS*)

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SUMMARY

Preliminary data are presented of the results of a prospectively randomized trial on elimination of gram-negative rods and yeasts from the bowel on the infection prevention in granulocytopenic patients. Only antimicrobial agents were used that do not affect the anaerobic flora, as they have an important function in preventing colonization with resistant microorganisms. One acquired infection was found in the decontaminated group against 19 acquired infections in the control group.

INTRODUCTION

Granulocytopenic patients have an increased risk of infections. These are mainly caused by gram-negative rods (2, 3, 6, 11-13, 18, 20). The majority of these pathogens come from the digestive tract (8, 9, 17), so that infection prevention should be directed against gram-negative bacteria at this site. These microorganisms can be eliminated by so called gut sterilization. However, this procedure is not without risk since massive overgrowth with

*)In: Fliedner et al (eds.): Clin. and Experim. Gnotobiotics. Zbl. Bakt. Suppl. 7 Gustav Fischer Verlag. Stuttgart · New York · 1979 p. 377-382.

resistant microorganisms may occur (10). The chances of unwanted colonization with pathogens can strongly be reduced by leaving the anaerobic fraction of the intestinal flora intact (14-16). Consequently it was investigated whether the incidence of gram-negative infections in granulocytopenic patients with acute myeloid leukaemia, acute non-myeloid leukaemia and granulocytopenia due to aplastic anaemia could be reduced by the prophylactic administration of antimicrobial agents which are not or only minimally effective against anaerobic bacteria such as nalidixic acid, co-trimoxazole or polymyxin B. Amphotericin B or nystatin were given to prevent yeast colonization in the gastrointestinal tract.

PATIENTS AND METHODS

Adult hospitalized granulocytopenic patients in whom a diagnosis was made of granulocytopenia due to bone marrow depression or acute myeloid leukaemia or acute non-myeloid leukaemia were admitted to the study when they had a granulocyte count below 1000/μl.
They were prospectively selected at random to receive either treatment aiming at a selective decontamination of the bowel or to serve as a control group.
A study period of a case was terminated at death, or on discharge from the hospital or when the granulocyte count was above 1000/μl three times in succession. If the patients were hospitalized more than once, each hospitalization period was considered to be one study period. All cases on study for less than seven days were considered not evaluable. The patients were treated on the open ward and supplied with the normal non-sterile hospital food. When indicated, the patients received supportive treatment: blood, leukocyte or platelet transfusions and intravenous broad-spectrum antibiotics.

Selective decontamination

Selective decontamination for gram-negative rods was performed with nalidixic acid (8 g/day) or co-trimoxazole (three times a day two tablets, each containing 80 mg trimethoprim and 400 mg sulfamethoxazole) or polymyxin B (800 mg/day). For selective elimination of yeasts amphotericin B suspension (2 g/day) or nystatin suspension (at least $6 . 10^6$ I.U./day) was used. With exception of co-trimoxazole the antimicrobial agents were divided into four portions. All drugs were given orally.

Microbiological surveillance

In both the selectively decontaminated and the control group routine cultures from the throat and the faeces were performed three times a week. In case of a suspected infection additional specimens like blood, urine and sputum were cultured. Blood cultures were taken when the axillary temperature rose above 38.5°C. All the specimens were only aerobically cultured on selective media.

Haematological surveillance

As the stratification into acute myeloid and acute non-myeloid leukaemia necessitated a rapid diagnosis, this differentiation was made by the peroxidase staining. Acute myeloid leukaemia was diagnosed when blast cells in the blood or in the bone marrow stained positive with peroxidase.
In granulocytopenias due to aplastic anaemias, the diagnosis was based on bone marrow biopsy as well as peripheral blood studies. White blood cells were counted by the Coulter counter (>3000 cells/μl) or in a counting chamber (<3000 cells/μl).
Absolute levels of granulocytes were calculated from differential counts and the total white blood cell count. Differentials were performed on 300 cells. Counting of granulocytes was repeated three times a week.

Clinical surveillance

Complete physical examination of the patient was performed on the day of randomization and repeated when the patient had fever. Additional investigations as X-rays of the chest, sinuses etc. were done when indicated.

Registration of infections

Infection was diagnosed when clinical signs and symptoms were present with or without microbiological documentation of the causative agent. If the axillary temperature was above 38.5°C, these infections were considered to be severe.

Statistical analysis

Statistical analysis of the infection rate was done with the chi-square test.

RESULTS

Patients

From the 64 cases admitted to the study, five could not be evaluted because the patients were followed for less than seven days. Relevant data concerning these remaining 59 cases are given in Table I.

Table I: Data about patients in 59 evaluable study periods.

	Benign granulocytopenia		Acute myeloid leukaemia		Acute Non-myeloid leukaemia	
	Selective decontamination	Control	Selective decontamination	Control	Selective decontamination	Control
Patients	12	12	7	9	8	7
Males/females	6/6	7/5	3/4	6/3	6/2	5/2
Average age	50.5	58	58	57	24.4	23
Cases	12	12	7	9	10	9

Microbiology

On three weekly examinations of faeces a definite reduction of gram-negative rods and yeasts was found in the faeces in the decontaminated group. No change was found in the presence of the *enterococci*. In throat swabs a reduction of gram-negative microorganisms was also found in the decontaminated group; an obvious decrease in the percentage of samples with positive yeast cultures was not observed.

Infections

In 10 study cases, severe infections were present on admission; all the other infections were acquired (Table II).

Table II: Distribution of severe infections in both of the groups.

	Selective decontamination	Control group	Total
On admission	4	6	10
Acquired	1	19	20
Total	5	25	30

In the decontaminated group only one infection, a *Candida* septicaemia, occurred (Table III). This patient was at that time unable to consume the oral antifungal treatment because of his debilitated clinical condition. Of all 19 severe infections in the control group, 13 were bacteriologically proven, six were unproven (Table III). Out of the 13 severe infections with

Table III: Severe acquired infections in both the decontaminated and the control group.

	Selective decontamination	Control group	Total
Periods of severe clinical infections without bacteriological documentation	0	6	6
Periods of severe clinical infections with bacteriological documentation	1	13	14
Total	1	19	20

bacteriological confirmation in the control group, eight were gram-negative, two were gram-positive and three were mixed (Table IV).
The difference in the number of acquired infections in the selectively decontaminated group and in the control group was significant ($P < 0.0005$).

Causative microorganisms

Data concerning the causative organisms of the acquired infections in the control group are given in Table IV. The localization and the nature of the

Table IV: Severe acquired infections with bacteriological confirmation in the control group.

	Gram-negative rod	*S. aureus*	Yeast or fungus	Mixed*	Total
Urinary tract	3				3
Lung	4	2	1	2	5
Blood	2	1			3
Pharynx	1				1
Anorectal region	1		1	1	1

* Lung: gram-negative rod plus fungus. Gram-negative rod plus *S. aureus*. Anorectal region: gram-negative rod plus yeast.

gram-negative rods are listed in Table V. In two cases only a gram-stain identified a gram-negative infection; one in sputum and one in multiple organs at autopsy.

Table V: Localization and nature of the causative gram-negative rods in the severe acquired infections in the control group.

	Urinary tract	Lung	Blood	Pharynx	Anorectal region	Total
E. coli	2				1	3
Klebsiella		2				2
Pseudomonas	1		1	1		3
Proteus		1				1
Gram-negative rod		1	1			2

DISCUSSION

Although the results are preliminary since the study is not yet completed, a definite reduction in the frequency of severe infections was observed in the selectively decontaminated group. Since most infections in granulocytopenic patients are known to be caused by gram-negative microorganisms existing in the digestive tract (2, 3, 6, 11-13, 18, 20), the effect of infection prevention by selective decontamination could be explained by the observed reduction of gram-negatives in the bowel and in the throat. No evidence of overgrowth with microorganisms was found in any decontaminated patient which we ascribed to the intact colonization resistance since the anaerobic flora was not affected. The regular bacteriological examination may also have contributed to this phenomenon because as soon as higher numbers of resistant microorganisms were found, the antibiotic regime for selective decontamination was changed accordingly. Oral administration of antifungal agents appeared to be more or less successful in suppressing colonization of the intestines with *Candida* species. In the oropharynx amphotericin B suspensions were less effective. For this site antifungal treatment requires improvement. Amphotericin B lozenges may be helpful in this respect (5).

In spite of the fact that in the first year of the study all patients were treated under open ward conditions and received the normal non-sterile hospital food, the daily contaminations acquired in this way did not result in

colonization with pathogenic bacteria in the digestive tract and if it occurred, it was not for more than a few days.
Because prevention of infection in selectively decontaminated patients appeared to be at least as successful as has been reported in the literature for total decontamination and isolation (4, 7, 10, 19), we favour this selective decontamination under open ward conditions, not only for the comfort of the patient, but also for economic reasons. It remains to be investigated whether some degree of isolation such als highly cooked food and sterile beverages improve the results.
A more extensive report of this study wil be published later when a larger number of patients in the subgroups has been randomized and studied.

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CHAPTER III

BACTERIOLOGICAL ASPECTS OF SELECTIVE DECONTAMINATION OF THE DIGESTIVE TRACT AS A METHOD OF INFECTION PREVENTION IN GRANULOCYTOPENIC PATIENTS*)

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ABSTRACT

The bacteriological results of a prospectively controlled clinical trial of selective decontamination of the digestive tract as a method of infection prevention in granulocytopenic patients are presented. Selective elimination of *Enterobacteriaceae*- and *Pseudomonodaceae* species was performed by the oral administration of nalidixic acid or co-trimoxazole or polymyxin. Yeasts were selectively eliminated by amphotericin B or nystatin. The choice of the drugs used in this study was based on their capacity of selective elimination of gram-negative rods and yeasts, without affecting the anaerobic part of the gut flora which is responsible for the colonization resistance.

As compared with the control group, the selectively decontaminated patients had significantly less ($P < 0.0005$) gram-negative rods and/or yeasts in their throat swab cultures and in their feces. This reduction may explain the clinical effectiveness of selective decontamination.

INTRODUCTION

Aerobic gram-negative rods and yeasts may cause life threatening infections in the granulocytopenic patient. In many reports the gastro-intestinal tract has been shown to be the reservoir of these potentially pathogenic

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microorganisms (3, 19, 20, 24, 38). Elimination of this source can result in a reduction of the infection frequency in this group of patients. This can be performed by the oral administration of non-absorbable antibiotics (like gentamicin and vancomycin) (total decontamination) (3, 7, 9, 13, 15, 16, 20, 23, 25, 28, 38). This procedure however, eradicates not only aerobic bacteria, but also the anaerobic part of the gut flora (5,20). Anaerobes, however, rarely cause infections in the granulocytopenic patient (1, 10, 18). On the other hand they may even have a beneficial influence in the prevention of infections caused by aerobic bacteria. The protective effect of anaerobes, in concert with the host, involves the control of the growth of aerobes. This mechanism which regulates the colonization pattern of the digestive tract – many details of which are not yet known – is called Colonization Resistance (CR) (32, 39).

In animal experiments it has been found that the number of anaerobes is important for the CR (40). Therefore, we have searched for a method of infection prevention which selectively eradicates aerobic gram-negative rods and yeasts keeping the anaerobic part of the gut flora intact. In animal studies this was realized (14, 35, 36) and the procedure was called Selective Decontamination of the Digestive tract (SDD). Selective decontamination is only feasible with antimicrobial drugs, which have one of the following properties:

1. a narrow spectrum, not affecting anaerobes (nalidixic acid, polymyxin, amphotericin B and nystatin).
2. a proportionally stronger cidal effect on aerobes than on anaerobes so that selective elimination of gram-negative rods can be attempted by careful dosing of the drug: co-trimoxazole (35) and neomycin (8, 12).

Based on the principle of conserving the CR we have developed a method for infection prevention which was aimed at selective elimination of aerobic gram-negative rods (using nalidixic acid or co-trimoxazole or polymyxin) and yeasts (using amphotericin B or nystatin) from the g.i.-tract, without affecting the CR-responsible anaerobes.

Recently we have described the results of a prospectively randomized study in 105 granulocytopenic patients in whom the prophylactic value of this selective decontamination of the digestive tract was investigated (27, 30, 31). In the 53 SDD-treated patients it resulted in a significant reduction of severe infections as compared with an equally sized control group which did not receive any prophylactic treatment (27).

We report here the bacteriological results of the 53 SDD-treated patients

as well as those of the 52 control patients. The success of selective decontamination and the effect of this procedure on the colonization resistance are discussed.

PATIENTS AND METHODS

Patients

All patients were adults and had acute leukemia (myeloid or lymphoid) or aplastic anemia. If they were granulocytopenic ($<1000/mm^3$) the patients were allocated at random either to receive selective decontamination of the digestive tract (SDD) or to serve as a control group not receiving prophylactic treatment. No isolation measures were taken in both groups; all patients were treated in normal bedrooms under open ward conditions and received non-sterilized hospital food. Patient characteristics are given in Table I. More details were described previously (27).

Table I: Patient characteristics

	SDD	Control
Number of patients	53	52
with acute myeloid leukemia	19	18
with acute lymphoid leukemia	16	15
with aplastic anemia	18	19
Weeks on study	209.5	192.0
with granulocyte count $<100/mm^3$	72.5	74.5
$100-500/mm^3$	74.5	61.5
$>500/mm^3$	62.5	56.0

Selective decontamination of the digestive tract

The choice of antimicrobial drugs for SDD was based on their influence on the CR. Animal experiments had shown that the drugs used in this study were safe in this respects (8, 14, 35, 36). This was later confirmed by Guiot and Van Furth (12) in man.
SDD was directed both at *Enterobacteriaceae*- and *Pseudomonodaceae* species and yeasts. *Staphylococcus*- and *Streptococcus* species were not

selectively eliminated in the study presently reported. For selective elimination of gram-negative rods a selection was made out of nalidixic acid (8 grams per day), co-trimoxazole (2880 milligrams, i.e. 480 mg trimethoprim and 2400 mg sulfamethoxazole per day) or polymyxin (800 milligrams per day). The susceptibility of the isolated gram-negative rods and known or presumed hypersensitivity of the patient determined which of the three drugs were administered for selective decontamination.
The addition of a second drug was considered indicated when during SDD-treatment resistant gram-negative rods were isolated from a patient. Decontamination of yeasts was performed with amphotericin B (2 grams per day) or nystatin (6.10^6 I.U. per day). All drugs for SDD were given orally as tablets with exception of amphotericin B which was administered as a suspension. The dose of co-trimoxazole was divided into three portions, all other antimicrobial drugs for SDD were administered four times a day. SDD-treatment was continued until the peripheral granulocyte count was above 1000/mm^3 of blood, measured three times in succession. Blood counts were performed thrice weekly. Other criteria for termination of a study period were death of the patients and discharge from the hospital.

Bacteriological surveillance

Because the patients were not isolated and therefore might become colonized by nosocomial resistant bacteria, the potentially pathogenic flora of the patients was monitored. Routine bacteriological monitoring was restricted to throat- and fecal specimens. Bacteria isolated from these samples reflected the potentially pathogenic microflora of the beginning and the end of the gastro-intestinal tract. All routine cultures were performed aerobically, three times a week, both in the SDD- as well as the control group. On the moment of randomization the first cultures were performed. Culturing of throat swabs and fecal samples was also performed during SDD-treatment. In (the few) patients who stayed in the hospital after termination of their study period, culturing was continued up to one week after stopping in order to get informed about the recolonization pattern.

Throat swabs

Throat swabs were cultured semi-quantitatively on sheep blood agar,

MacConkey (MC) agar (Oxoid), Yeast Isolation (YI) agar (Merck), Levinthal agar (Oxoid) and in Brain Heart Infusion (BHI) broth (Oxoid) as well. After overnight incubation at 37°C the BHI broth culture of the throat swab was subinoculated on blood-, MC-, YI- and Levinthal agar.

Feces

From one gram of feces a serial dilution was made in BHI broth, which was subsequently incubated at 37°C during 18 hours. Thereafter, the dilutions with "growth" were subinoculated on MC-, YI- and kanamycin-aesculin-azide (KAA) agar (Oxoid).
In this way the concentration of the aerobic gram-negative rods, yeasts and enterococci could be determined. Anal swabs – occasionally used in case no feces were available – were processed in the same semi-quantitative way like the throat swabs. They were streaked onto MC-, YI- and KAA agar and in BHI broth for enrichment. All solid media, except MC and KAA were incubated for at least 42 hours at 37°C.

Identification and biotyping of *Enterobacteriacea*- and *Pseudomonodaceae* species

Pure cultures were made of all different looking colonies of gram-negative rods. They were identified according to the API 20E system (4, 21). Each API 20E profile number was called a biotype. Biotypes isolated from the samples from the patient at admission, were referred to as endogenous, biotypes isolated later on were called exogenous. "Common" biotypes were those biotypes which were most frequently isolated from the throat swabs and the fecal specimens from all the patients (SDD and control) during the whole study period.

Susceptibility to antimicrobial drugs

The susceptibility pattern of all biotypes, *Staphylococcus aureus* and *beta-hemolytic streptococci* was determined by agar diffusion (2) on Diagnostic Sensitivity Test (DST) agar (Oxoid) with sensitivity test tablets (Neosensi-tabs, Rosco).
The susceptibility of *Enterobacteriaceae*- and *Pseudomonodaceae* species was tested to the drugs used for SDD: nalidixic acid, co-trimoxazole and

polymyxin. Moreover, the susceptibility was tested for a number of drugs currently in use for the treatment of infections.

Monitoring the colonization resistance

To monitor the effect of SDD on the CR we have measured the concentration of the *enterococci* in the feces (37).

Statistical analysis

Statistical analysis was done by the chi-square (two sided).

RESULTS

Patients

One hundred and thirteen patients were randomized; 8 were excluded for evaluation because the period of selective decontamination was less than 7 days. From the 105 evaluable patients, 53 had been allocated to SDD and 52 to the control group not receiving any prophylactic antimicrobial treatment. At the moment of randomization there was no difference in the number of patients with potentially pathogenic bacteria in the throat swab cultures (Table II) and in the feces (Table III) between the SDD- and the control group.

Table II: Culturing results of the throat swabs.

	SDD	Control	
Number of patients	53	52	
Number of patients at randomization with:			
gram-negative rods:	13	13	
yeasts:	26	27	
gram-positive cocci:			
(*S. aureus* + *Str. pyogenes*):	14	10	
Number of cultures (one week after randomization):	432	388	
% of cultures with:			
gram-negative rods:	3.7	29.6	P <0.0005
yeasts:	27.3	42.6	P <0.0005
gram-positive cocci:			
(*S. aureus* + *Str. pyogenes*):	7.2	9.0	

Table III: Culturing results of the feces.

	SDD	Control	
Number of patients	53	52	
Number of patients at randomization with:			
gram-negative rods:	53	52	
yeasts:	14	11	
enterococci:	52	51	
Number of cultures (one week after randomization)	406	325	
% of cultures with:			
gram-negative rods:	25.6	95.1	P <0.0005
yeasts:	8.4	21.5	P <0.0005
enterococci:	91.4	92.6	

Selective decontamination of the digestive tract

The majority of the patients received oral amphotericin B for suppression of growth of yeasts; only four patients were treated with 6.10^6 I.U. of nystatin per day during 46 days in total. For selective elimination of gram-negative rods, co-trimoxazole and nalidixic acid were most frequently used (Table IV), respectively during 711 days (29 patients) and 602 days (36 patients). In 12 patients both drugs were used for different reasons (resistant strains, side effects). These two drugs were never given in combination. Changing to or addition of polymyxin to one of the former drugs was done in the case of isolation of resistant *Enterobacteriaceae-* or *Pseudomonodaceae* species from two or more consecutive fecal samples. In 6 patients polymyxin was added for this reason for SDD; this drug was used during 149 days in total (Table IV).

Bacteriological surveillance

Throat swabs

At the moment of randomization *Enterobacteriaceae-* and *Pseudomonodaceae* species and/or yeasts were cultured from the throat swabs

Table IV: Distribution of the drugs for selective decontamination of the digestive tract in the 53 patients. Readjustment of treatment was performed because of side-effects such as drug allergy or nausea, or because of the isolation of resistant gram-negative rods.

Diagnosis	Number of patients treated for selective decontamination by			
	single drugs		two or more drugs	
			consecutively	consecutively or in combination
	nalidixic acid	co-trimoxazole	nalidixic acid and co-trimoxazole	nalidixic acid and/or co-trimoxazole and/or polymyxin
acute myeloid leukemia	5	6	6	2
acute lymphoid leukemia	9	3	2	2
aplastic anemia	9	3	4	2
all patients	23	12	12	6

from respectively 13 and 26 patients in the SDD-group (Table II) *Haemophilus influenzae* was in no case isolated from the throat swabs. Excluding the first week of study, gram-negative rods and/or yeasts were cultured from 3.7, respectively 27.3% of the throat swabs from the SDD-patients. The incidence of these microorganisms in the throat of SDD-patients was significantly reduced as compared with the control group (Table II). SDD appeared not to influence the number of cultures with *Staphylococcus aureus* and *Streptococcus pyogenes* (Table II).

Feces

All patients had gram-negative rods in their initial fecal cultures. After starting nalidixic acid treatment, the fecal cultures became negative (i.e. gram-negative rods could not be cultured) after an average period of 6.9 $\pm$ 3.9 days; for co-trimoxazole this period was 8.9 $\pm$ 4.6 days. In patients

who received polymyxin, the first fecal sample obtained thereafter was negative; i.e. the SDD-effect of this drug was achieved in one to three days. In 15 patients SDD was completely successful: all the fecal samples obtained after the first negative fecal sample remained free of gram-negative rods during a treament period of 208 days in total (range 4-32 days).

In 13 patients no or only one negative fecal sample could be obtained because of the short observation period of less than 14 days (reason for termination: granulocytes $>1000/mm^3$, death or discharge from the hospital). In the other 25 SDD-patients gram-negative rods were cultured from one (14 patients) or more (11 patients) fecal samples during SDD-treatment.

The 406 fecal samples obtained in the SDD-group after the first week of study were compared with the 325 in the control group. The percentage of fecal cultures with gram-negative rods differed significantly between both groups (95.1 versus 25.6) (Table III).

Yeasts were cultured from the initial fecal samples from 14 SDD-patients. After starting the antifungal SDD, these microorganisms disappeared in 4.1 ± 3.9 days from the feces. Thereafter alle the fecal samples obtained in 39 patients remained free of yeasts. In the other 14 patients, yeasts were cultured from one (8 patients) or more fecal samples. From all the specimens from SDD-patients – omitting those obtained during the first week of each study period – yeasts were cultured in 8.4%. This implied a significant reduction in comparison with the percentage of fecal samples with yeasts in the control group (Table III).

Identification and biotyping of *Enterobacteriaceae*- and *Pseudomonodaceae* species

In the stool specimens of the 25 SDD-patients which were positive for *Enterobacteriaceae*- and *Pseudomonodaceae* species in total 108 API 20E-biotypes were isolated after the day on which the first negative fecal sample had been obtained. Usually the gram-negative rod was found in a low concentration in these samples (Figure 1) and one API 20E type at the time; occasionally more than one biotype was found in a sample.

To evaluate whether gram-negative rods were eliminated or only suppressed by SDD-treatment, 121 biotypes – isolated during (108) and after (13)

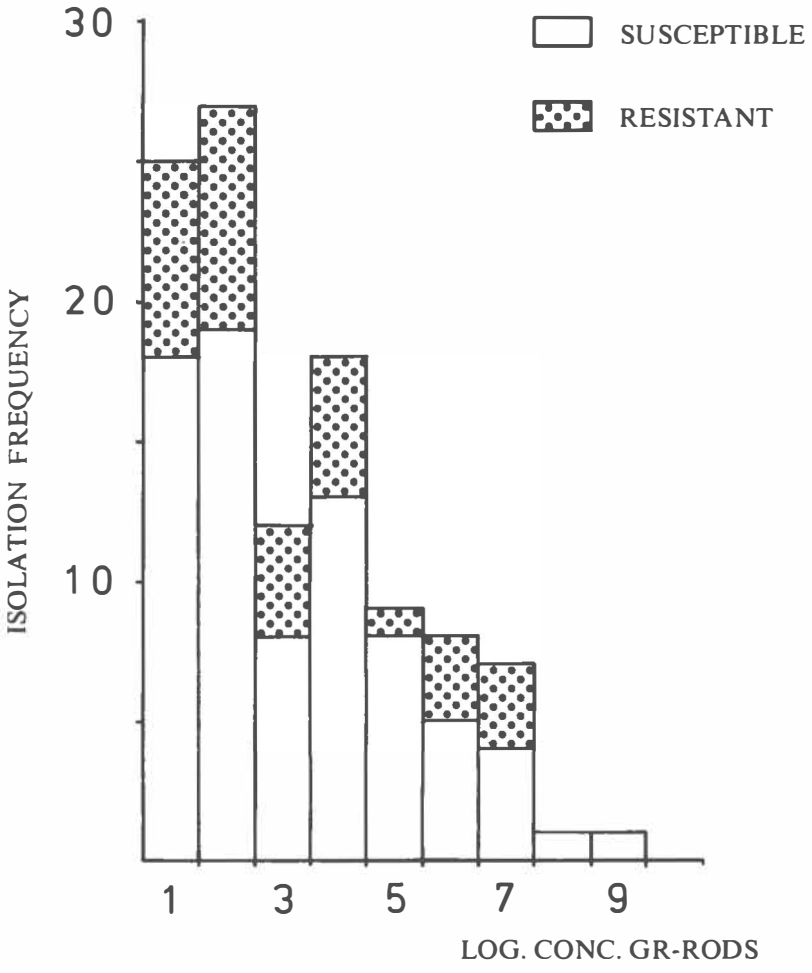


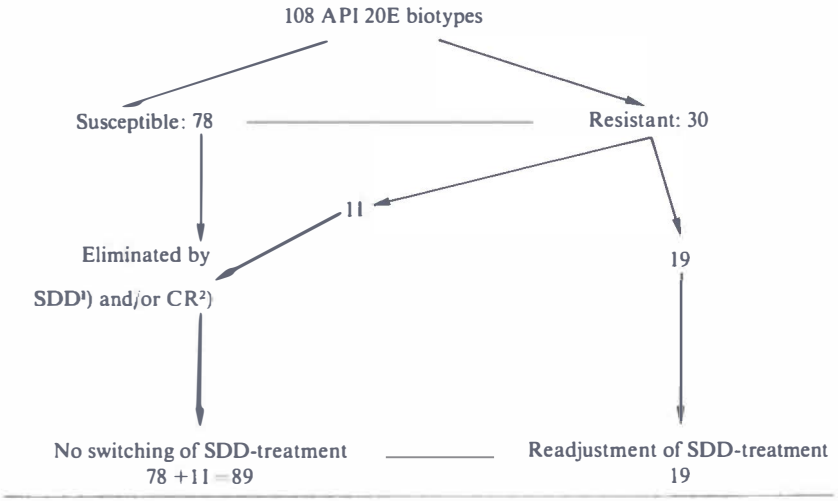
Figure 1: Frequency of occurrence and concentration of gram-negative rods in the feces during selective decontamination after the first negative fecal sample.

the period of SDD – were compared with those isolated on admission. Of these 121 biotypes 93 were exogenous (77%), i.e. nosocomial in origin. Of the 28 biotypes which also had been isolated at admission, 16 (57%) were "common" and of the 93 exogenous biotypes mentioned above, 22 (24%) were "common". "Common" biotypes although also isolated at admission, could have been nosocomial in origin.

Susceptibility pattern of *Enterobacteriaceae*- and *Pseudomonodaceae* species

Of the 108 biotypes, isolated during SDD and after the first negative fecal sample had been obtained, 78 were susceptible for the antimicrobial drug used for SDD, used on the moment of occurrence of the positive culture. In no case they could be cultured from the next fecal sample. The same applied for 11 out of 30 remaining biotypes which were resistant to the drugs used for SDD. For elimination of 19 of the 30 resistant biotypes readjustment of the treatment was necessary. This was either because the gram-negative bacterium persisted for a longer period in the patient's flora or because it was found in a high concentration in the fecal cultures (Table V).

Table V: Susceptibility of 108 API 20E profiles (biotypes) of *Enterobacteriaceae*- and *Pseudomonodaceae* species – isolated after the first negative fecal sample – applied as a guideline for readjustment of selective decontamination treatment in the 53 patients.



[1]) Selective decontamination of the digestive tract
[2]) Colonization Resistance

Colonization resistance

Regarding the preservation of the C R the concentration of the *enterococci*

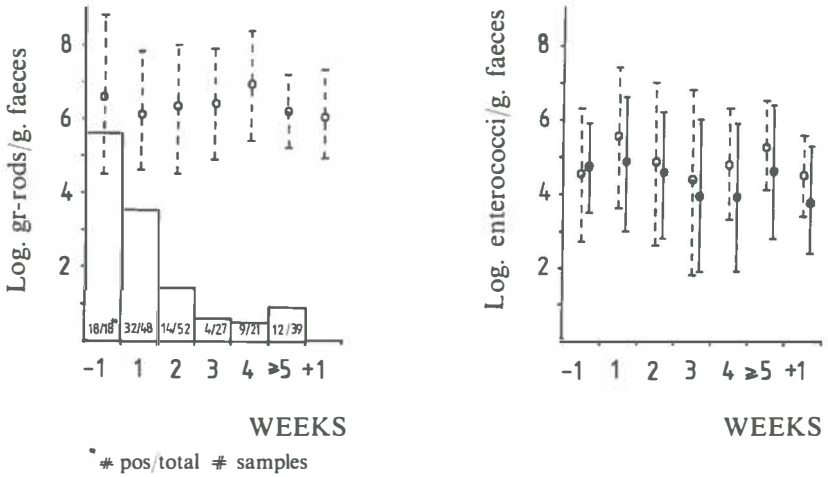


Figure 2: Concentration of gram-negative rods and *enterococci* in the feces of selectively decontaminated (——) and control (- - - -) patients. Samples included, were derived from patients in the period during which they did not receive systemic antimicrobial therapy in addition.

and the aerobic gram-negative rods in the feces was compared between the SDD- and the control group (Figure 2). Because other antimicrobial agents then the SDD-drugs, given to the patients in the case of a (suspected) infection, might influence the CR (29, 33), only those samples were included for this evaluation which were obtained during the periods without systemic antimicrobial therapy.

While the concentration of gram-negative rods decreased rapidly after the start of SDD, the concentration of the *enterococci* (naturally resistant to the drugs used for SDD) did not change.

DISCUSSION

The results of the present study indicate that with nalidixic acid or co-trimoxazole or with polymyxin it appeared possible to eliminate selectively susceptible *Enterobacteriaceae*- and *Pseudomonodaceae* species from the gastro-intestinal tract. Fecal cultures became free of gram-negative rods

one week after the start of nalidixic acid or co-trimoxazole treatment. In patients who were treated with polymyxin this was achieved in a few days. The rapid effect of polymyxin is in accordance with the results of De Gast en Van Saene who treated *Salmonella* carriers successfully with this drug (11).

As all patients had aerobic gram-negative rods in their feces at admission (Table III), the bacteriological effect of SDD could best be measured by frequent monitoring of the fecal flora. In this way the moment of disappearance of *Enterobacteriaceae*- and *Pseudomonodaceae* species from the feces and the maintenance of this condition could be determined rather accurately. Because it took about one week of treatment before the fecal samples from the SDD-patients became free of gram-negative rods and yeasts, the samples obtained after the first week of study were compared in both groups. Gram-negative rods could still be cultured from 25.6% of the fecal samples in the SDD-group. This was probably due to the fact that our patients recieved non-sterile food and were in no way isolated. Although the food was not sampled routinely for bacteriological investigation during the study period, there is evidence form the literature that food is a main source of *Enterobacteriaceae*- (6, 22) and *Pseudomonodaceae* (22, 26) species. The (possible continuous) oral contaminations with small numbers of gram-negative rods from food, beverages and other environmental sources resulted sometimes in positive fecal- and throat specimens. However, in no case colonization (persistance of the gram-negative bacterium in the gastro-intestinal tract) for more than a week was observed.

It was remarkable that there was also a significant reduction in the number of throat swabs with gram-negative rods, despite the short presence of the swallowed SDD-drugs in the oropharynx. Of both absorbable SDD-drugs (especially co-trimoxazole) a fraction may have been excreted in the saliva and therewith may have added to the SDD-effect in the oropharynx.

Yeasts were more frequently found in the throat (Table II) than in the feces (Table III). At the moment of randomization about half of the cases had yeasts in their oropharyngeal cultures (Table II); this figure was about 25% for the feces. After starting (non-absorbable) amphotericin B administration the number of throat swabs and fecal samples with yeasts decreased significantly. After the first negative fecal sample following the onset of treatment, 8.4% of the subsequent fecal cultures revealed yeasts; this could be due to contamination from the (positive) oropharynx, for yeasts could still be cultured from 27.3% of the throat swabs. This was a

remarkable reduction in comparison with control group patients if one realizes that the drug has only a short stay and therewith a short contact time in the oropharynx. This reduction can not be explained by an additional systemic effect since amphotericin B as well as nystatin are non-absorbable drugs.

The bacteriological efficacy of SDD, i.e. the reduction in the number of aerobic gram-negative rods and yeasts is in accordance with the clinical effect of this treatment as described previously (27, 30).

Biotyping of *Enterobacteriaceae* species has been used for epidemiological purposes (34). In our study this technique suggested that with SDD we were perhaps eliminating gram-negatives rather than suppressing them, since the majority of the biotypes isolated during and after the period of SDD-treatment differed from the those isolated at admission. Of the 28 biotypes isolated both at admission and during treatment 16 were "common". This means that about half their number was ubiquitary which makes it difficult to draw conclusions about their endogenous or exogenous origin.

The biotypes which were isolated during the SDD-treatment period after the first negative fecal sample had been obtained, were in general (72%) susceptible to the drugs used at the time they were isolated. Moreover, 11 out of 30 resistant biotypes disappeared without readjustment of the treatment, possible as a consequence of an intact CR. To which degree the CR was maintained intact during SDD, however, was difficult to estimate. Anaerobes of various species are apparently involved and for an optimal CR many of these species are required (40). Because of the complexity of anaerobic culturing and species determination we have made no attempt to perform an anaerobic inventory of the feces.

In rodents an enlarged cecum (17) and an increased concentration in the oropharynx as well as in the feces ("bacterial overgrowth") of an orally administered resistant bacterium (37) both indicate a decreased CR. These parameters, however, can obviously not be used in man. Therefore the concentration of the *enterococci* which are naturally resistant to the drugs used for SDD and which could be cultured from practically every fecal sample, were used as the next best parameter for the CR. During SDD the concentration of the *enterococci* remained stable and their concentration did not differ from those in the control group (Figure 2). In addition, most of the transient gram-negative rods found during SDD-treatment in the "positive" fecal cultures, disappeared without readjustment of the treat-

ment. From these two findings it could be concluded that the CR was not greatly disturbed. This intact CR we believe to be of great significance in the apparent effectiveness in the prevention of infections by SDD without isolation procedures. The need for nursing in a protective environment appears definitely decreased if one takes care of maintaining the CR. However, because in the conventional hospital environment the patients may continuously become contaminated by (small numbers of) potentially pathogenic bacteria – among which resistant strains could be present – frequent bacteriological monitoring is considered a prerequisite in this kind of infection prophylaxis.

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CHAPTER IV

SELECTIVE DECONTAMINATION OF THE DIGESTIVE TRACT IN GRANULOCYTOPENIC PATIENTS: THE OCCURRENCE OF RESISTANCE*)

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INTRODUCTION

Patients with haematological diseases associated with granulocytopenia are susceptible to frequent and serious infections (1-5). Most of these infections are caused by Gram-negative microorganisms which belong to the patient's endogenous bacterial flora (6-8) or have been acquired from the hospital environment, thereafter colonizing the patient's digestive tract (6, 9, 10).

Infection prevention can be accomplished by reducing the number of potentially pathogenic microorganisms in the gastrointestinal tract by means of oral nonabsorbable antibiotics, with or without a protected environment (7, 11-15). A disadvantage of the antibiotic decontamination of the gastrointestinal tract is the enhanced acquisition of resistant strains or the development of resistance to the oral antimicrobial drugs used for decontamination (11, 16). The acquisition of resistant bacteria is especially threatening when resistance to penicillin derivatives, aminoglycosides or other systemically applicable antibiotics is concerned.

We have recently reported the results of a prospective study on "selective decontamination" of the digestive tract (SDD) in granulocytopenic patients (17, 18). In this study a selectively decontaminated group of patients was compared with a control group. Nalidixic acid or co-trimoxazole was given in order to reduce the number of potentially pathogenic micro-

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organisms without affecting the anaerobic flora which is apparently responsible for the integrity of the colonization resistance (19). In the selectively decontaminated patients, the overall frequency of serious infections (bacteriologically confirmed and clinical) was reduced by 75%.
For this paper, we have evaluated the occurrence of resistance to nalidixic acid or co-trimoxazole as well as the influence of systemic therapy with gentamicin and carbenicillin on the acquisition of Gram-negative bacteria and the sensitivity pattern of these microorganisms in both the selectively decontaminated patients and patients of the control group.

PATIENTS AND METHODS

Patients

Since January, 1977, all adult patients with granulocytopenia due to bone marrow failure, acute myeloid leukaemia, acute non-myeloid leukaemia or remission induction treatment have been randomized to either a SDD or a control group. The control group was obviously not treated with antimicrobial drugs for SDD. Patients were entered into the study when their peripheral granulocyte count was below 1000/mm^3. Systemic antibiotics were given in both groups when clinical signs and symptoms of infection were present with or without bacteriological confirmation. Systemic antimicrobial therapy consisted of gentamicin (3 × 80 mg/day i.v.) and carbenicillin (30 g/day i.v.) as the first choice; only occasionally were other systemic antibiotics used. The few patients thus treated were excluded from evaluation.
All patients, those selectively decontaminated as well as the control group, were treated under standard conditions in conventional hospital ward rooms containing 4 beds. They were supplied with the normal non-sterile hospital food and treated by the same group of physicians.

Selective decontamination

SDD for Gram-negative bacteria was performed with nalidixic acid (8 g/day), with co-trimoxazole (2 tablets thrice daily) or with colistin (800 mg/day). The later drug was given only occasionally and then in combina-

tion with nalidixic acid or co-trimoxazole. With the exception of co-trimoxazole, the daily dosage of the antimicrobial drugs was divided into 4 doses. All drugs were given orally. The occurrence of drug side-effects, such as nausea, and the sensitivity pattern of the potentially pathogenic bacteria isolated from the patient's flora determined the choice of the antimicrobial drugs used for SDD.

Bacteriology

In both the SDD group and the control group (also during systemic antimicrobial therapy) cultures of the oropharynx swabs and faeces were performed routinely thrice weekly. The oropharynx cultures in the SDD-treated group, however, were generally negative for species of the *Enterobacteriaceae* family and therefore the results were not evaluated. In the control group Gram-negative bacteria could be isolated in 27% of the oropharynx swabs. The stools were cultured quantitatively with special attention for aerobic Gram-negative rods. *Enterobacteriaceae* species were identified and biotyped according to the API 20E system (20, 22). For all different biotypes, the sensitivity patterns were determined for the following antimicrobial drugs: Nalidixic acid- co-trimoxazole, colistin, gentamicin, cefalotin, ampicillin and carbenicillin. Sensitivity was tested with antimicrobial sensitivity test tablets using the agar diffusion method (Kirby-Bauer) (23). Strains were considered sensitive or resistant according to the criteria presented in Tabel I.

Table I: Criteria for sensitivity and resistance (inhibition zone in mm).

	Sensitive	Resistant
Nalidixic acid	≥28	≤23
Co-trimoxazole	≥28	≤23
Polymyxin	≥22	≤18
Gentamicin	≥28	≤23
Cefalotin	≥26	≤22
Ampicillin	≥26	≤22
Carbenicillin	≥26	≥22

Statistical analysis

Differences in the sentivity patterns were analysed statistically by the χ^2 test.

RESULTS

In evaluating the occurrence of resistant Gram-negative bacteria, the faecal flora of 73 patients was analysed. Patients were divided into 4 groups.

Control patients (Group A)

In 23 patients the sensitivity pattern of the faecal flora was tested. During a study period of 76 weeks, a total of 177 samples was obtained. The sensitivity pattern was determined for 215 biotypes of Gram-negative bacteria. More than 85% of the biotypes was sensitive to nalidixic acid, co-trimoxazole, colistin and carbenicillin.
Occasionally resistance was found to cefalotin (22%) and ampicillin (18%) (- (Fig. 1). Intermediate sensivity was not evaluated; however, it concerns only a small number of biotypes.

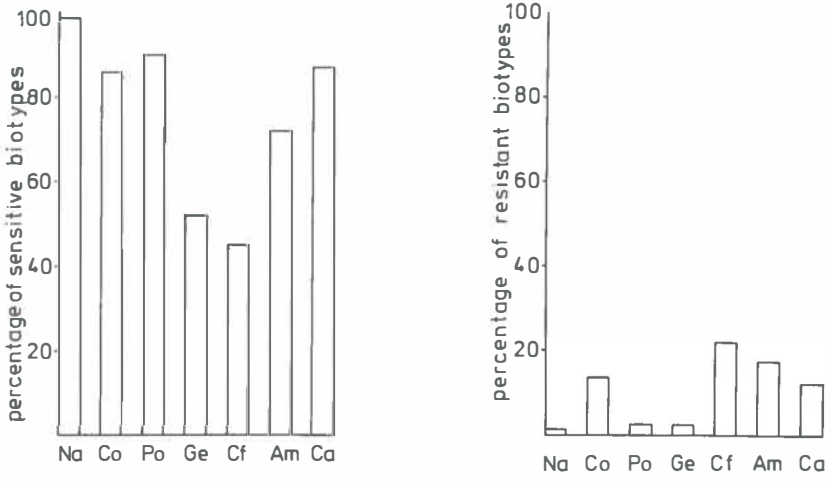

Fig. 1: Percentage of sensitive and resistant biotypes (N = 215) isolated from 23 patients without SDD and without systemic treatment (Na = nalidixic acid; Co = co-trimoxazole; Po = polymyxin; Ge = gentamicin; Cf = cefalotin; Am = ampicillin; Ca = carbenicillin).

SDD patients not receiving systemic antibiotics (Group B)

Co-trimoxazole: 18 patiens were studied during 57 weeks of hospitalization and SDD treatment. Results of sensitivity testing are given separately for each subsequent week of treatment (Fig. 2). A total of 100 biotypes was studied. The number of biotypes rapidly decreased when the effect of SDD became evident in the faecal flora. This implied a reduction of approximately 36% (19/53) in the number of biotypes after the first week of SDD. No increase in resistance to co-trimoxazole was found during treatment. The sensitivity of the faecal flora to the other agents tested was not influenced either. An occasional resistant biotype was found after the fourth week of study. However, because the number of biotypes isolated during that period was small, we felt that their percentage could not be evaluated.

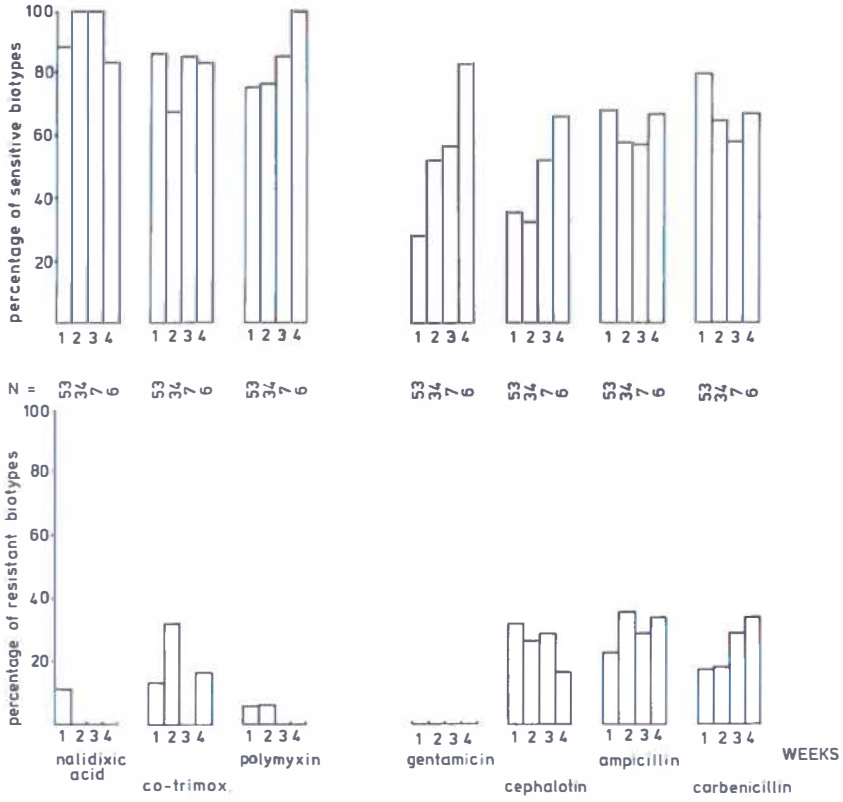

Fig. 2: Percentage of sensitive and resistant biotypes isolated from 18 patients treated with SDD (co-trimoxazole) without systemic antibiotic therapy.

Nalidixic acid: 20 patients were studied for 50 weeks. The sensitivity patterns were determined in 135 biotypes. In the second week of treatment, resistance to nalidixic acid was seen in 7 of 25 biotypes (28%) (Fig. 3). In the following weeks of nalidixic acid treatment, however, these biotypes could no longer be isolated. No influence on the sensitivity pattern of the other antimicrobial drugs was found (Fig. 3). The reduction in the number of biotypes in the first week of treatment was approximately 77% (85/110) (Fig. 3).

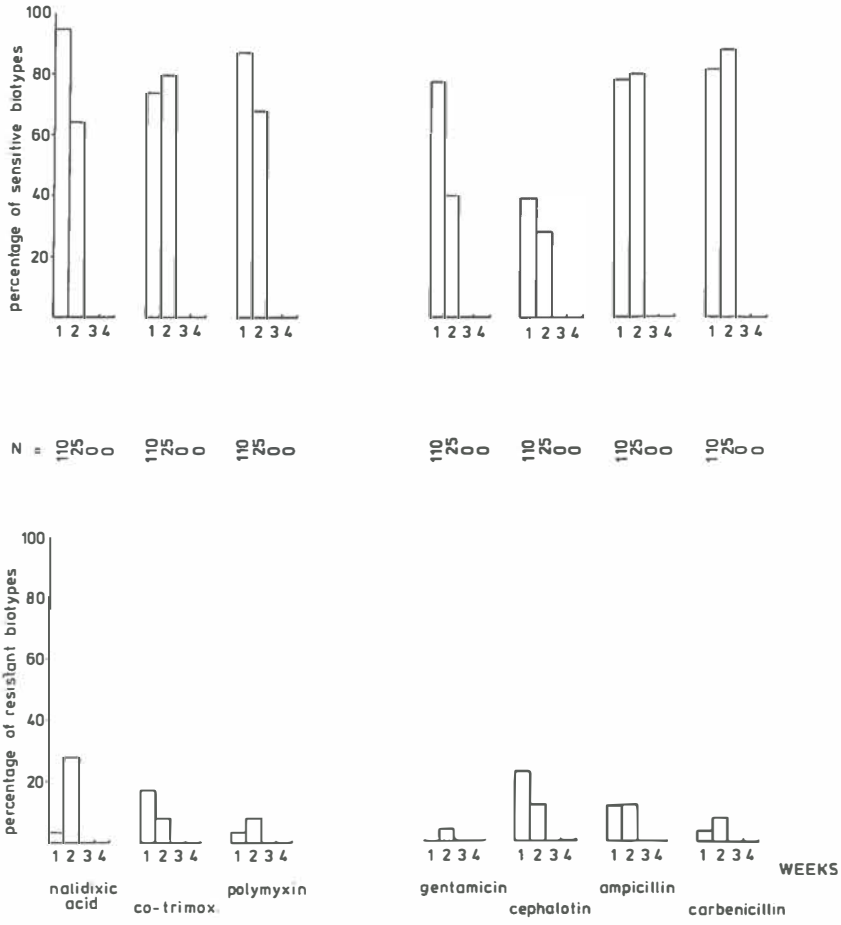

Fig. 3: Percentage of sensitive and resistant biotypes isolated from 20 patients treated with SDD (nalidixic acid) without systemic antibiotic therapy.

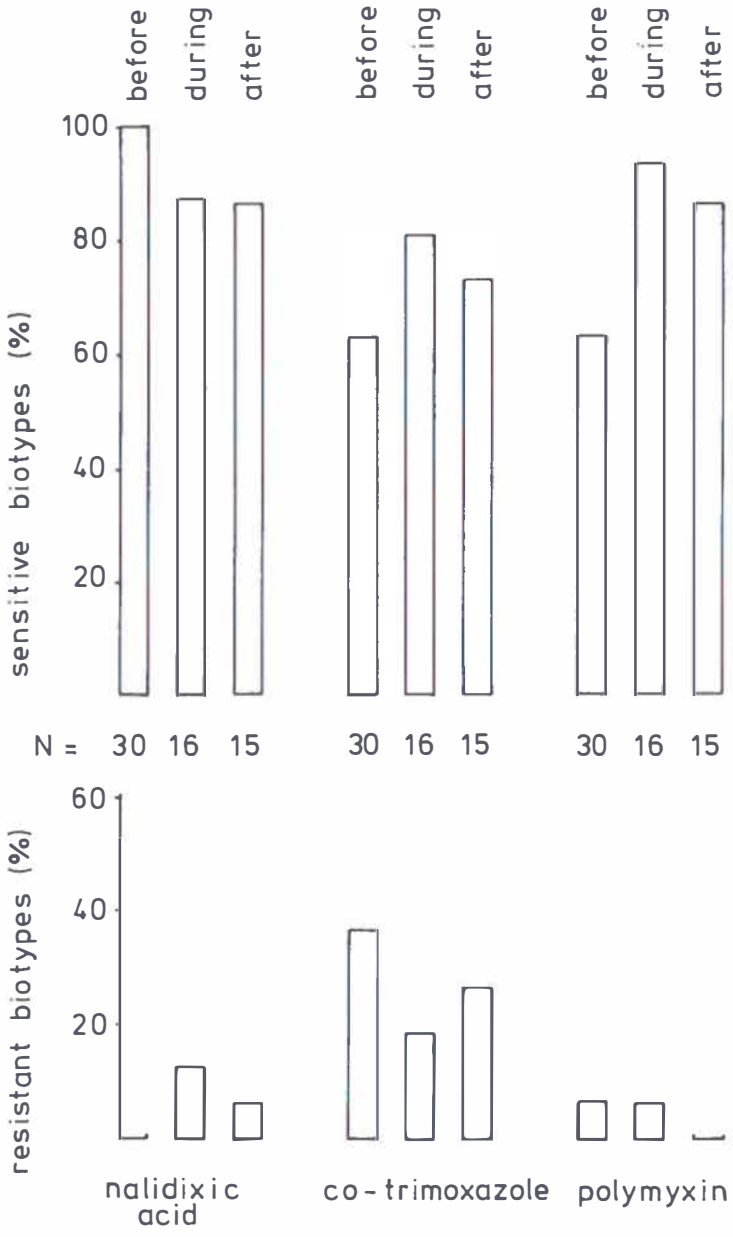

Fig. 4a: Percentage of sensitive and resistant biotypes isolated from 10 SDD-treated patients 2 weeks before, 2 weeks during and 2 weeks after therapy with gentamicin and carbenicillin; sensitivity pattern of nalidixic acid, co-trimoxazole and polymyxin.

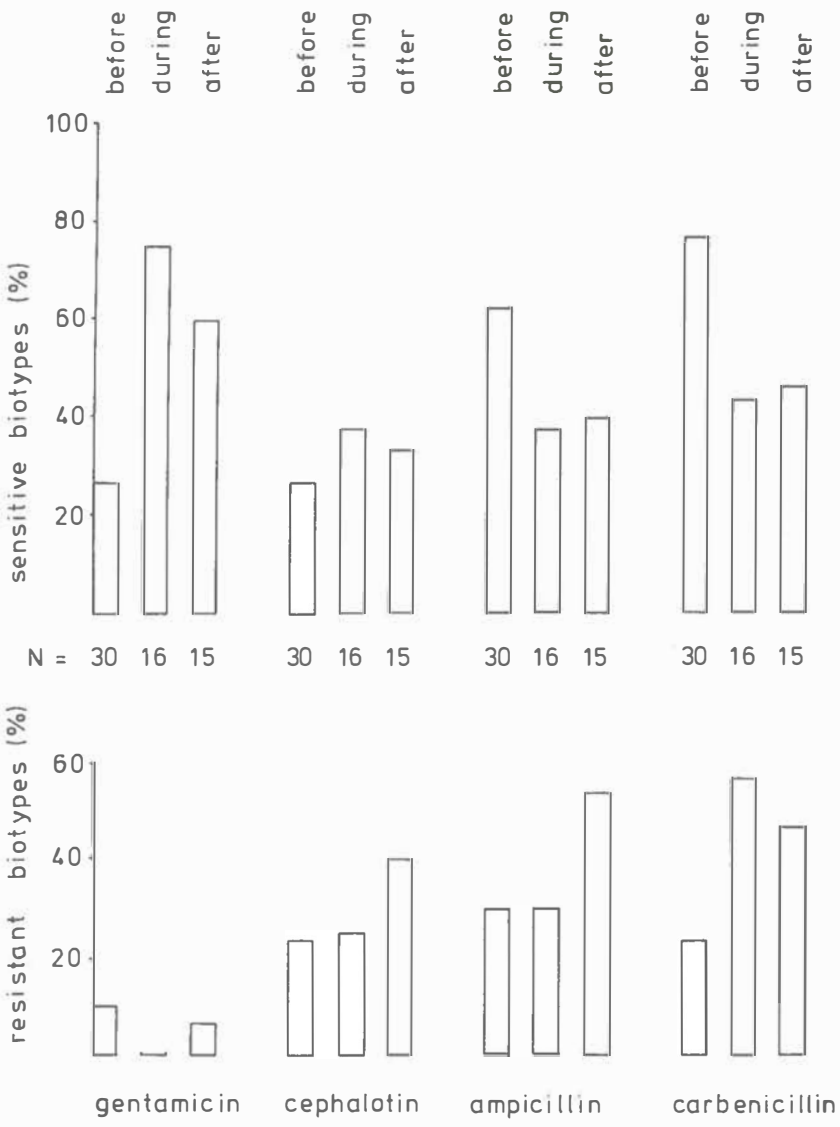

Fig. 4b: Percentage of sensitive and resistant biotypes isolated from 10 SDD-treated patients 2 weeks before, 2 weeks during and 2 weeks after therapy with gentamicin and carbenicillin; sensitivity pattern for gentamicin, cefalotin, ampicillin and carbenicillin.

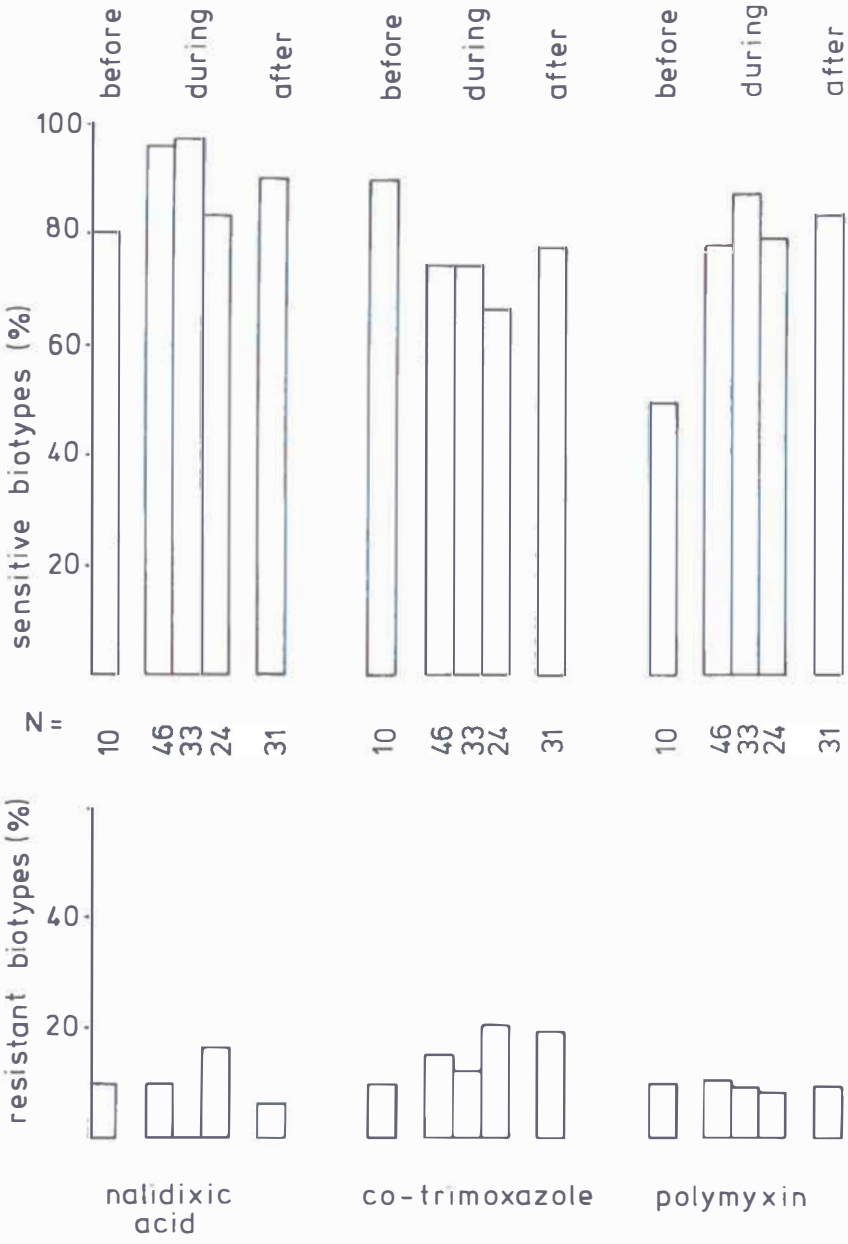

Fig. 5a: Percentage of sensitive and resistant biotypes isolated from 12 patients without SDD, but treated with gentamicin and carbenicillin for 3 weeks; sensitivity pattern for nalidixic acid, co-trimoxazole and polymyxin.

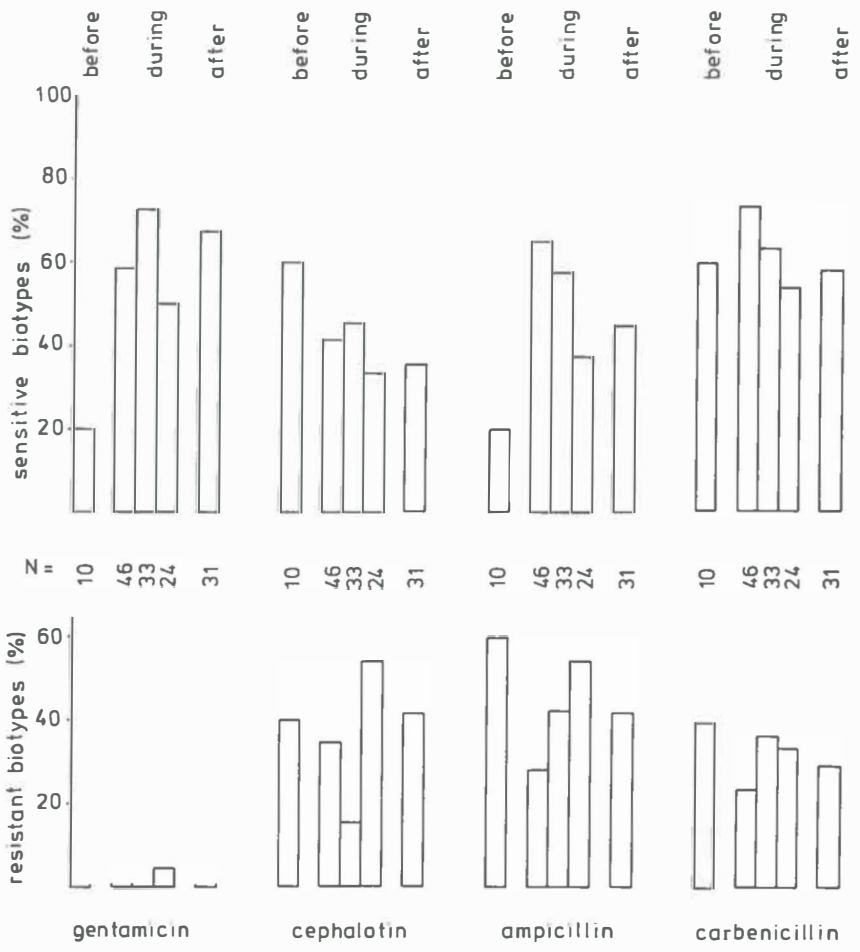

Fig. 5b: Percentage of sensitive and resistant biotypes isolated from 12 patients without SDD, but treated with gentamicin and carbenicillin for 3 weeks; sensitivity pattern for gentamicin. cefalotin, ampicillin and carbenicillin.

SDD patients receiving therapeutic systemic antibiotics (Group C)

Ten patients were studied; a total of 16 biotypes was isolated and tested for sensitivity. Results of sensitivity testing of biotypes isolated during the 2 weeks preceding systemic antimicrobial treatment, during 2 weeks of treatment and 2 weeks thereafter, are presented in Figure 4. For SDD

nalidixic acid or co-trimoxazole was used; for systemic treatment gentamicin and carbenicillin were given for 2 weeks. No increase of resistance to SDD drugs was found (Fig. 4a). The increase of resistance to cefalotin, ampicillin and carbenicillin was not statistically significant (Fig 4b).

Patients receiving therapeutic systemic antibiotics without SDD (Group D)

In 12 patients 144 biotypes were tested for their sensitivity pattern. Systemic antibiotic therapy consisted in all cases of a combination of gentamicin and carbenicillin; these drugs were given for 3 weeks (in contrast to the previous group). Results of sensitivity testing are again given for biotypes isolated before, during and after therapy (Fig 5). No resistance developed to the therapeutically used antibiotics (Fig. 5b) or to the other antimicrobial drugs (Fig. 5a).

Acquisition of new biotypes during systemic treatment

In the systemically treated control group, particularly in the third week, the number of biotypes isolated per patient had almost doubled in comparison with the baseline value before systemic treatment (Table II). In the SDD-treated group such an increase was not observed: on the contrary, the number of biotypes was greatly reduced. It must be realized, however, that this group was only treated with systemic antibiotics for 2 weeks whereas the control group was treated for 3 weeks.

Table II: Number of biotypes per number of patients before, during and after systemic treatment with or without SDD (group C and group D).

	Group C	Group D
Before	30/7	10/3
During	16/10	46/12 (first week)
		33/9 (second week)
		24/4 (third week)
After	15/10	31/6

DISCUSSION

During treatment with nalidixic acid or co-trimoxazole for selective decontamination of the gastrointestinal tract, we did not observe an increase in resistance to these antimicrobial drugs. In the second week of treatment a temporary increase of strains resistant to nalidixic acid was seen (Fig. 3). There was, however, only a small increase in the absolute number of resistant strains. Consequently, the increase in the percentage of resistant biotypes may have been due to a decrease in the absolute number of sensitive strains. The resistant strains, however, were presumably dealt with by colonization resistance (19) as the resistant Gram-negative bacteria all disappeared in the subsequent week. Since oral colistin administration resulted (in the few patients who were so treated) in a rapid (within a few days) disappearance of sensitive Gram-negative rods, combined initial treatment in the first week with nalidixic acid should be considered for improvement of SDD.

During therapy with nalidixic acid for Gram-negative urinary tract infections, resistance to this drug is apparently relatively easily induced; this has been found in other-studies in 7-25% of patients thus treated (24-25). The relatively high incidence of resistance during therapy of urinary tract infections is probably caused by the (lower) dosages of <4 g of nalidixic acid per day. Since the drug is not completely absorbed in the gastrointestinal tract, favourable conditions for the induction of resistance (i.e. concentrations just above the minimum inhibitory concentration of the intestinal *Enterobacteriaceae)* may occur in the lower gut during this dose regimen. Stamey and Bragonje, applying 4 g of nalidixic per day, found only 7% resistance to the causative microorganisms in the urine (24); in the faecal flora they found 11% resistance. The faecal concentrations of biologically active nalidixic acid in the intestines after 8 g per day, as applied in this study, are probably considerably higher. The fact that all sensitive *Enterobacteriaceae* were eliminated from the faecal flora may explain why, except for a passing exogenous Gram-negative biotype, increased resistance was not seen. In other words, chances of induction of resistance to nalidixic acid may have been reduced in accordance with the reduction of the total number of Gram-negative bacteria during selective decontamination.

The lack of development of resistance to co-trimoxazole is completely in accordance with the literature (26, 27). It can therefore be concluded that nalidixic acid and co-trimoxazole used in high doses in SDD do not

increase the risk of development of resistance to these drugs or influence the sensitivity pattern of the flora to current systemic antibiotics (gentamicin, carbenicillin, cefalotin and ampicillin).
The development or acquisition of gentamicin-resistant strains varies widely (28-31). A rather high prevalence of resistance has been reported after oral gentamicin therapy (30) or topical application of gentamicin (31). Furthermore, the presence of multi-resistant Gram-negative bacteria, particularly in a hospital, is important for the acquistion of these resistant bacteria in the digestive tract. The finding of a very low number of acquired resistant strains in our patients may reflect the low number of gentamicin-resistant strains in the haematological department of our hospital. Acquistion of biotypes of Gram-negative bacteria continued during treatment with gentamicin and carbenicillin. The total number of biotypes during the third week of systemic treatment in the control group of patients even appeared to increase without an increase of biotypes resistant to these antimicrobial drugs. The same, i.e., no noticeable increase in resistance, applies to cefalotin as well as to ampicillin.
In conclusion: We did not see an increase in resistance of potentially pathogenic bacteria to nalidixic acid or to co-trimoxazole in the stools of selectively decontaminated granulocytopenic patients. If strains resistant to these drugs were found, they were most likely acquired while the intact colonization resistance of the digestive tract apparently prevented colonization by these microorganisms.

SUMMARY

We recently reported the favourable results for infection prevention in granulocytopenic patients of selective decontamination of the digestive tract (SDD). The orally administered antimicrobial drugs used for this purpose were nalidixic acid, co-trimoxazole or colistin.
These drugs were selected because they do not influence the colonization resistance of the digestive tract. In this study, the development of resistance in the Gram-negative faecal flora of the patients to these antimicrobial agents was investigated. We found no increase of resistance to nalidixic acid or co-trimoxazole during SDD with these agents aimed at infection prevention. Furthermore, the influence of systemic treatment with gentamicin and carbenicillin for infections on the development and acquisition

of resistance of the faecal flora was analysed in a control group of patients as well as the SDD group in the first week of treatment. We found no clear evidence of resistance to gentamicin. No significant increase in resistance to carbenicillin was noted either.

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CHAPTER V

AMPHOTERICIN B CONCENTRATIONS IN SALIVA AFTER APPLICATION OF 2% AMPHOTERICIN B IN ORABASE*)

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SUMMARY

2% amphotericin B in orabase® was applied to the buccal mucous membranes of healthy volunteers. Amounts of 1 g and 0.5 g were used. The experiments were performed during the day time as well as during the sleeping hours at night. At several intervals after application saliva samples were collected and the amphotericin B concentrations were determined. Application of 1 g of orabase® with 2% amphotericin B appears to maintain a sufficiently high concentration for four hours, 0.5 g for three hours. Application of these amounts before going to sleep guarantees a sufficient concentration until awakening the next morning.

ZUSAMMENFASSUNG:

Amphotericin B Konzentrationen im Speichel nach einer Anwendung von 2% Amphotericin-B im Orabase. 2% Amphotericin B im Orabase® wurde auf die Rachenschleimhaut gesunder Freiwilliger appliziert. Dabei kamen Mengen von 1 g bzw. 0,5 g zur Anwendung. Die experimente wurden während des Tages und während der Nacht durchgeführt. Mehrmals nach der Anwendung wurden Speichelproben gesammelt und die Amphotericin B Konzentrationen bestimmt. Die Applikation von 1 g der Orabase® von 2% Orabase von 2% Amphotericin B scheint eine ausreichend hohe Antibiotika Konzentration für vier Stunden zu geben, 0,5 g für drei Stunden. Die Anwendung dieser Mengen vor dem Schlafengehen garantiert eine ausreichende Konzentration bis zum nächsten Morgen.

*) In: Infection 6 : 16-20, 1978

INTRODUCTION

The number of patients with a reduced resistance to infection (e. g. patients with aplasia, bone marrow malignancies, transplants, or under therapy with steroids and/or cytostatics) has been increasing during the last years. The problems of treatment of the often severe infections in this group of patients have also increased. Most of the infections are caused by gram-negative rods, a smaller number by yeasts and fungi (1, 2, 3, 4). In the cases in which *Candida* species cause infection, treatment is generally very difficult as antifungal drugs for systemic use are mostly static and often toxic at higher dose levels. In the oropharynx particularly, *Candida* infections tend to spread. Therefore, in the case of such infections, fungistatic antibiotics should, in addition, be applied locally to limit further expansion of the process. Prevention of spread could best be attained with a suitable antimycotic drug which reaches sufficient concentration in the saliva. This method of approach was tested and appeared to be most effiently realized by applying the antimycotic drug in a concentration of 2% in orabase® (5). Orabase® is a protective paste which adheres to mucous membranes and moist skin surfaces.

For optimal effect, the antimycotic drug should be active at low concentrations. Moreover, it should be palatable and chemically stable in the saliva. Finally, this local antimycotic treatment should not induce resistance in the microorganisims and should not sensitize the patient (6, 7).

These considerations led to the decission to use amphotericin B in orabase® for this purpose. An additional advantage of this drug combination is that it is generally available.

The part of the study reported here should answer the question concerning dosage of this drug combination. It is thought that optimal cooperation by the patient has a positive influence on the effect of the treatment. For this reason the study was performed in healthy volunteers rather than in patients.

Before use of the antimycotic drug, and at several intervals thereafter, a saliva sample was collected and the amphotericin B concentration determined. In this way, the period was determined during which a sufficient concentration (higher than the minimal inhibitory concentration (MIC) of most *Candida* species) was maintained.

Orabase® was also administered to volunteers during the sleeping hours at night in order to study the amphotericin B concentration overnight.

MATERIALS AMD METHODS

Volunteers

A group of 19 healthy volunteers was subdived into four groups and treated as described in Table I. In Groups 1 and 2 the procedure was repeated seven to ten times by each volunteer and eight times in Groups 3 and 4.

Table I. Treatment and dosages of orabase® with 2% amphotericin B used in 19 different volunteers (some volunteers participated in more than one group).

Volunteers and methods	Day time		Sleeping hours at night	
	Group 1	Group 2	Group 3	Group 4
Number of volunteers	9	8	5	4
Dose of orabase® in grams	1	0.5	1	0.5
Total number of orabase® applications	85	79	40	32
Minutes before and after application of orabase® when saliva samples were collected	−1	−1	−1	−1
	1	1	—	—
	15	15	15	15
	30	30	—	—
	60	60	—	—
	120	120	—	—
	180	180	—	—
	240	240	—	—
			475±43	478±48

During the day-time experiments, which lasted four hours, no eating, drinking or smoking was permitted. The saliva samples were deep frozen and stored at -20°C as soon as possible after collection. Only after each

volunteer had completed his/her whole series, were the amphotericin B concentrations determined in all the samples at the same time. Because of the differences in the consistency of the saliva (which might influence the outcome of the titration) each sample was titrated four times in total (twice on two different days).

Orabase® Treatment

Orabase® with 2% amphotericin B* was stored at 4°C. Shortly before use amounts of 1 g or 0.5 g were distributed to the volunteers. The orabase® was then applied by the volunteers themselves to their gums, using a finger.

Determination of amphotericin B concentration in the saliva and preparation of stock solutions.

To determine the amphotericin B concentrations, the biological dilution method (modification of Cimijotti's method) (8) was chosen. The test was performed in microtrays. 50 mg of the active substance of amphotericin B ad perfusionen was dissolved in 5 ml of sterile demineralized water. This stock solution was then stored at -20°C until required for the preparation of the various dilutions used for the determination of a reference line. The latter consisted of eight dilutions decreasing in concentration from 500 μg/ml to 3.9 μg/ml and were prepared fresh weekly.

Reference line and amphotericin B concentration in the saliva.

For the preparation of the reference line, eight known amphotericin B concentrations were serially diluted 1 : 2 in microtrays previously filled with 0.05 ml of brain heart infusion (BHI) broth (Difco) containing 0.5 mg of cephaloridine per ml of BHI. Cephaloridine was added to the BHI to inhibit multiplication of the normal bacterial flora in the saliva, which would contaminate the cups.

This amount of 0.05% cephaloridine was found not to influence the position and the slope of the reference line. After serial dilution 0.05 ml of a diluted fresh overnight culture of a sensitive *Candida albicans* strain (about 10^6 viable cells per ml) was added to each cup. Thereafter, the trays were covered with transparent tape and incubated at 37°C for 18 hours.

* Orabase® with 2% amphotericin B (Fungilin®) was kindly provided by Squibb-Nederland. Composition of orabase®: Gelatine B. P. 16.7%; Pectine 16.7%; Sodium Carboxymethylcellulose 16.7%; Polyethyleen av. Mol. Wt. 32,000 2.5%; Paraffin Liquid. B. P. 47.4%.

The reference line was determined by plotting the number of cups with inhibited growth against the logarithm of the corresponding amphotericin B concentration. The results of the 31 titrations of the test strain gave a range of values for the MIC which varied from 0.2-0.8 μg/ml (mean 0.41 μg/ml, SD 0.24).
Amphotericin B concentrations in the saliva were determined by the same micromethod as described above for the reference line. Instead of amphotericin B suspensions with known concentrations, saliva samples were serially diluted. A new reference line was made on each day on which amphotericin B concentrations in saliva were determined.

Influence of the saliva on the amphotericin B concentration:
This was investigated by "checkerboard titration" of the saliva and a solution of amphotericin B with a known concentration of 31.25 μg/ml in microtrays.

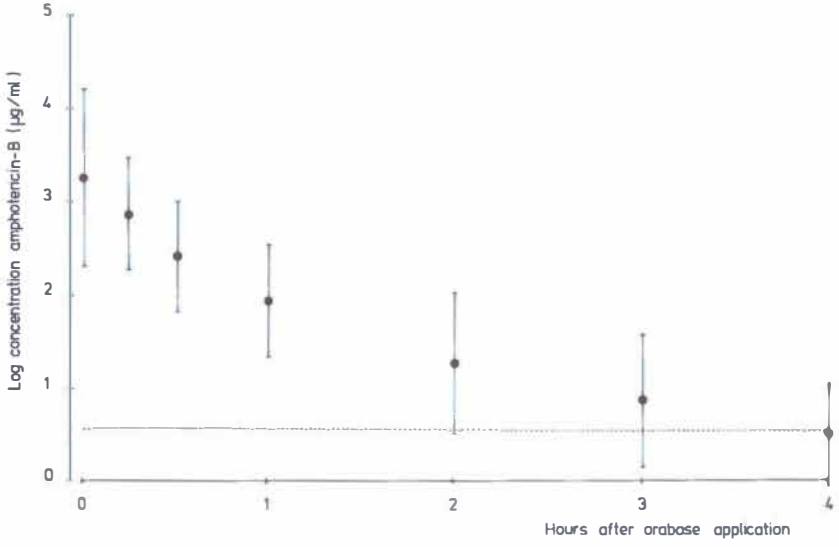

Figure 1 : Amphotericin B concentrations (mean and SD) in saliva at different intervals after application of 1 g of orabase® with 2% amphotericin B.

RESULTS

Amphotericin B concentrations in the saliva.

The results of treatment with 1 g of amphotericin B 2% in orabase® during the day-time or overnight are shown in the Figures 1, 2, 3 and 4. The means and the standard deviations of the amphotericin B concentrations shown at different intervals following application of orabase® were calculated from all the values obtained in the volunteers of the corresponding group. The horizontal dotted line represents a concentration of 3.7 μg/ml. This is the highest MIC of *Candida* species for amphotericin B found in the literature (7, 9).

From the Figures 1 and 2 it is apparent that after application of 1 g or 0.5 g of amphotericin B orabase® during the day-time, the amphotericin B concentration in the saliva is sufficient for four and three hours respectively.

After application of these amounts during the sleeping hours at night, the amphotericin B concentrations were found to be virtually undecreased by the following morning.

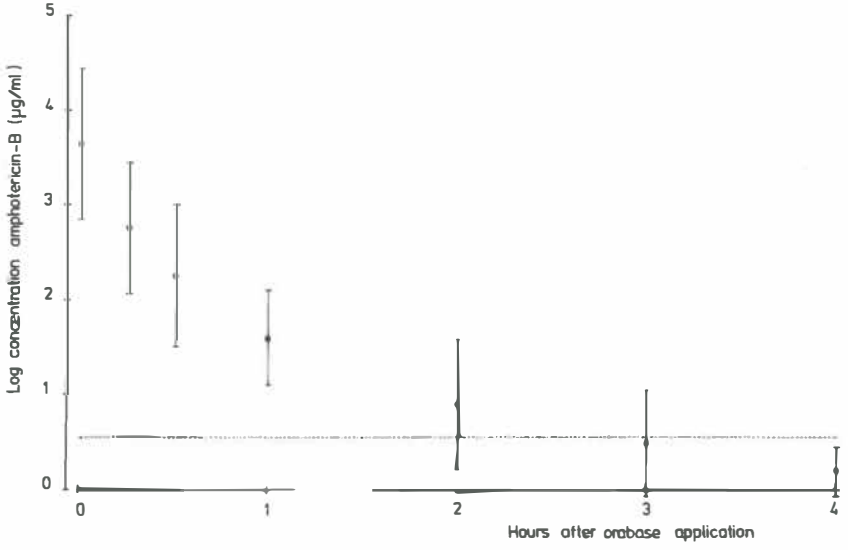

Figure 2: Amphotericin B concentrations (mean and SD) in saliva at different intervals after application of 0.5 g of orabase® with 2% amphotericin B.

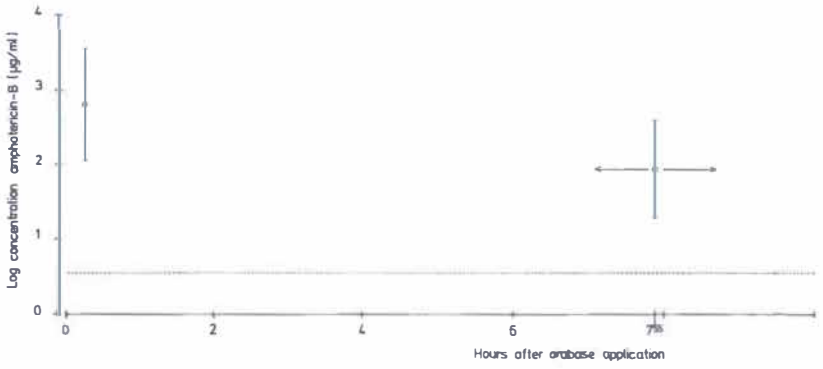

Figure 3: Amphotericin B concentrations (mean and SD) in saliva after application of 1 g of orabase® with 2% amphotericin B during the sleeping hours at night.

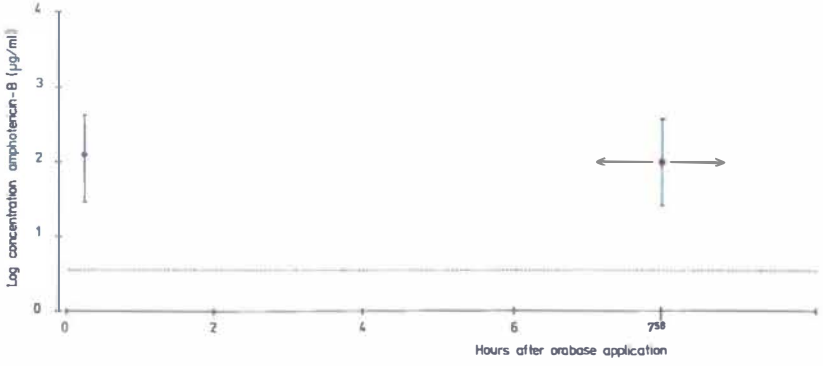

Figure 4: Amphotericin B concentrations (mean and SD) in saliva after application of 0.5 g of orabase® with 2% amphotericin B during the sleeping hours at night.

Concentration of "inhibitory substances" in the saliva samples before orabase® applications.

In a small number (13 out of 236) of the saliva samples taken before orabase® apllication, some inhibition of the growth of the test strain was observed. However, as this was an inconstant finding even in the 13 positive samples, it was presumed to be due to a technical error.

Influence of saliva on the MIC of the *Candida albicans* test strain for amphotericin B as determined by checkerboard titration.
In the saliva samples of 35.5% of the volunteers no change in the MIC of the *C. albicans* test strain was found.
In 64.7% a weak inhibiting influence was seen, which increased the MIC of the test strain by one dilution step (1 : 2).

Toxic side-effects:

Of the total 236 times that orabase® amphotericin B was administered to the 19 volunteers, vomiting, nausea and spasm of the gut were reported as possible side-effects of the treatment only five times. These adverse effects occurred in three volunteers.

DISCUSSION

This investigation indicates that it is possible to maintain "*Candida*-static" concentrationts of amphotericin B in saliva for a few hours. With repeated use this is presumably long enough for an efficient suppression of an oral *Candida* colonization. It is possible that differences may exist between healthy volunteers and patients with regard to the amphotericin B concentrations in the saliva following orabase® application. However, as long as there is orabase® on the mucous membranes in the oropharynx, *Candida* multiplication in the saliva will be inhibited. Taking 4 μg of amphotericin B per ml of saliva as minimal effective concentration, our results indicate that following application of 1 g of orabase® with 2% amphotericin B a sufficiently high amphotericin B concentration can be maintained for four hours (Figure 1). 0.5 g of orabase® with 2% amphotericin B may guarantee inhibition of growth for three hours following application (Figure 2). This means that for constant suppression a new dose of orabase® amphotericin B should be applied after that interval. These values furthermore indicate that there is a considerable safety margin in the dosage. If, for example, 1 g of orabase® is prescribed, this provides a wide safety margin for spillage or spoilage by the patient, and does not have serious consequences for the duration of a sufficient amphotericin B concentration in the saliva.
Furthermore, it can be expected that immediately after application of orabase® a noticeable reduction of the number of yeasts will occur when

amphotericin B 2% in orabase® is used to prevent *Candida* colonization in the oropharynx. In the absence of a local infection, subsequent use of this antimycotic drug in orabase® after each meal may cause further reduction of the number of yeast cells until complete suppression. In both groups of volunteers treated with 1 g and 0.5 g of orabase® respectively during the sleeping hours at night, the amphotericin B concentration in the saliva remained sufficiently high (Figure 3 and 4). The time during which the orabase® adheres to the mucous membranes may depend on a number of factors, such as the place of application, the number of movements of the tongue and the jaws and the amount of saliva produced. The more saliva produced and the greater the activity of the jaw, the more rapidly the orabase® is removed. This could explain why a high amphotericin B concentration is maintained during the sleeping hours at night, when all these factors are reduced.

Because all these factors also control the oropharyngeal population, suppression or multiplication of yeasts by amphotericin B suspended in orabase® during the sleeping hours at night may be of more importance than suppression during the day-time.

Our findings could also be of practical value when suppression of other colonizing microorganisms (bacteria) from the oropharynx is desired. If for example a suitable antimicrobial agent, suspended in orabase®, is applied in the oral cavity, an active concentration can be expected in the saliva for several hours (5, 10). This kind of suppressive antimicrobial treatment is presumably also most effective during the sleeping hours at night; i.e. more effective than when it is used at three or four hourly intervals during the day.

Summarizing, it seems plausible that application of 2% amphotericin B in orabase® three times a day after each meal, and particularly shortly before going to sleep, may provide an important method of preventing colonization and therewith infections in the oropharynx of patients with locally or generally decreased resistance of infection.

In addition, it may be of value as supportive therapy in existing infection in the oropharynx, especially in the prevention of spread of that infection to other places inside the gastrointestinal tract.

ACKNOWLEDGEMENTS

We gratefully acknowledge the excellent techincal assistance of Heleen Beyes and Rudi Tonk.

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CHAPTER VI

SALIVARY CONCENTRATIONS OF AMPHOTERICIN B FOLLOWING ITS USE AS AN ORAL LOZENGE*)

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SUMMARY

Amphotericin B concentrations were measured in the saliva of ten healthy volunteers who had sucked on lozenges with this drug. It appeared that high amphotericin B concentrations can be achieved in this way in the saliva. Even half an hour after swallowing the last remnant of a lozenge, the amphotericin B concentration was found to be high enough to suppress the growth of sensitive *Candida albicans* strains. The possible usefulness of amphotericin B lozenges in the selective decontamination of the oropharynx of yeasts and other fungi is discussed and compared with the application of this drug in orabase®.

ZUSAMMENFASSUNG.

Amphotericin B Konzentrationen im Speichel nach Anwendung als lutschtabletten. Die Amphotericin B Konzentrationen wurden im Speichel von zehn gesunden freiwilligen Versuchspersonen gemessen, die Tabletten mit dieser Substanz gelutscht hatten. Es stellte sich herhaus, daβ im speichel hohe Amphotericin B Konzentrationen erzielt werden können. Sogar eine halbe Stunde, nachdem der letzte Rest einer Lutschtablette geschluckt worden war, wurden Amphotericin B Konzentrationen gefunden, die hoch genug waren, um das Wachstum empfindlicher *Candida albicans* Stämme zu hemmen. Die Anwendbarkeit von Amphotericin B Lutschtabletten zur selektiven Dekontamination des Oropharynx von Hefen und anderen Pilzen wird diskutiert und mit der Anwendung der Substanz in Orabase® verglichen.

*) Infection. 8 : 63-65, 1980

INTRODUCTION

Granulocytopenia is a predisposing factor for infections (1, 2). Many of these infections are caused by gram-negative rods (1, 2, 3, 4); yeasts and other fungi however, may also lead to infectious complications (1, 3, 5, 6, 7). *Candida* lesions in the oropharynx are seen frequently, not only in the patient with a decreased immune capacity, but also during treatment with systemic antibiotics (8, 9, 10, 11, 12). In healthy individuals *Candida albicans* has been isolated from the blood stream after oral contaminations with high numbers (13). In the immune compromised host, lower concentrations of yeasts in the gastrointestinal tract may results in fungemia. Furthermore, antibiotic therapy may enhance multiplication of *Candida* species in the digestive tract of these patients and thus promote invasion (14). Yeasts and other fungi can be cultured from the oropharynx in a great number of the hospitalized patients (15). In about 50% of a group of 53 leukemic patients yeasts were cultured from the throat swabs. Oral treatment with an amphotericin B suspension gave some reduction in the number of positive throat cultures in these patients. There was also a reduction in the number of clinically observable and mycologically documented *Candida* lesions (in preparation). The use of local amphotericin B treatment may improve these results. High concentrations of amphotericin B can be realized by the application of this drug in orabase® (16).
The fact that the latter is a sticky paste and has an unpleasant taste however, makes it less suitable for long term use in cooperative patients. Recently amphotericin B lozenges became available for clinical studies. Others have used this application form with success in the treatment of oral thrush (17). In order further to investigate its applicability, particularly regarding preventive treatment, we have determined the concentrations of amphotericin B in the saliva in ten healthy volunteers at different intervals during and after the use of amphotericin B lozenges.

MATERIALS AND METHODS

Volunteers:

Ten healthy volunteers participated in the experiment. Each took one lozenge and tried to keep it in the mouth as long as possible. The

experiment was repeated ten times by each volunteer. In the first series of five experiments – each performed on a different day – the experiment lasted two hours; in the second series of five experiments, it lasted three hours. If the lozenge had dissolved within the alloted time interval, the moment of disappearance of the last remnant was recorded.
After two or respectively three hours, the rest of the lozenge was expelled and the dry weight was determined. For the determination of the amphotericin B concentration in the saliva, samples were taken before and at 15,30, 60 minutes etc. after the lozenge was put in mouth (Figure 1). Saliva samples were also taken 10, 20 and 30 minutes after swallowing or expelling the lozenge. During the experiment, drinking, eating and smoking were not permitted. Saliva samples were stored at -20° C until processing.

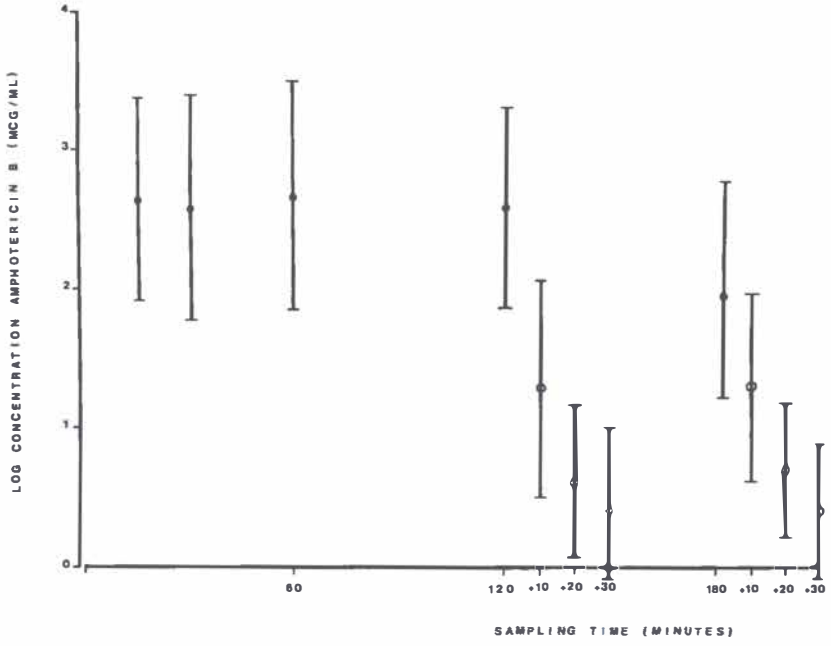

Figure 1: Mean and standard deviation of the concentration (expressed as ^{10} logarithm) of amphotericin B in the saliva at different times during the use of amphotericin B lozenges (●) and after dissolution or expulsion of the lozenges (O).

Amphotericin B lozenges*

Lozenges of about one gram (1.02-1.03 g), each containing 10 mg of amphotericin B, were used. They were stored at room temperature. Volunteers tried to keep the lozenge in their mouth as long as possible and without chewing during the experiment.

Determination of the amphotericin B concentration in the saliva samples.

Because we were interested in the biologically active amount of amphotericin B in the saliva samples, the concentration was determined according to a modification of the method of Goss and Cimijotti (18).
The test was performed in microtrays, previously filled with 0.05 ml of Brain Heart Infusion (BHI) broth (Difco), in which twofold dilutions of the saliva samples were made. Therafter 0.05 ml of a sensitive freshly diluted *C. albicans* strain (about 10^6 viable cells per ml) was added. The trays were covered with a transparent tape and incubated for 18 hours at 37°C. By comparing the number of cups without growth of the yeast strain in the diluted saliva samples with the inhibition of growth in the eight different concentrations of the reference line, the actual amount of amphotericin B in the saliva samples could be determined. Cephaloridine was added to the BHI (0.5 mg/ml) to inhibit the multiplication of the bacterial flora in the saliva which could contaminate the cups. This concentration of cephaloridine did not influence to any extent the viability of the test strain.

Reference line:

50 mg of the active substance of amphotericin B ad perfusionem was dissolved in 5 ml of sterile demineralized water. From this stock solution, which was stored at -20°C, eight different amphotericin B concentrations were made, varying from 500 μg/ml to 3.9 μg/ml (twofold dilutions). They were freshly prepared weekly. From each of the eight known concentrations twofold dilutions were made in microtrays. Measuring the growth inhibition of the added *C. albicans* strain gave the reference line.

*) Kindly provided by Squibb – Nederland.

RESULTS

All participants completed the first part of the study in which the lozenge was kept in the mouth for a maximum of two hours. In total, 50 lozenges were used. In 41 cases a part of the lozenge could be expelled after the two hour interval. This remnant of the lozenge was then collected and the dry weight was determined (mean: 0.28 g; range 0.03-0.76 g). In nine cases the lozenge had been dissolved within the two hours. This occurred four times in three volunteers; in one person the lozenge disappeared in all cases within the observation time of two hours. Because of nausea, one of the volunteers refused to participate in the second part of the experiment, which lasted three hours. In the second part of the study in which the lozenges were kept in the mouth for three hours, a total of 45 lozenges were used. After three hours remnants of lozenges could be collected for dry weight determination in 27 cases (mean: 0.20 g; range 0.01-0.67 g). The other 18 lozenges had dissolved in the mouth within an average interval of 105.8 minutes (SD 36.8). The concentration of the biologically active amount of amphotericin B was determined in the saliva samples collected at different intervals after the intake of the lozenge (Figure 1). Ten, 20 and 30 minutes after the two and three hour experiment i.e. after swallowing or expelling the lozenge, the amphotericin B concentrations in the saliva were also determined (Figure 1). As long as the lozenge was present in the mouth, the amphotericin B concentration in the saliva was found to be well above the minimal inhibitory concentration (MIC) of most *C. albicans* strains, i.e. about 4.0 μg/ml (19). This means that it may have been sufficient to suppress the growth of sensitive *C. albicans* strains in vivo. This supposition holds also for an interval of about 30 minutes after expelling or swallowing the lozenge.

DISCUSSION

The results of these experiments indicate that long-lasting inhibiting concentrations of amphotericin B can be achieved in the saliva with lozenges. As long as the lozenge was present in the mouth of the volunteers, the amphotericin B concentration in the saliva was found to be well above the MIC of most strains of *C. albicans*.

Even half an hour after swallowing the last remnant of a lozenge, *Candida*-static amphotericin B concentrations were found in the saliva. The mean amphotericin B concentrations found presumably represent better the actual concentration in the mouth than the SD values.
Peak concentrations of 1 mg/ml and more found in our study can only be explained by the presence of a small particle of the lozenge in the saliva sample investigated. Most of the volunteers were able to keep the lozenge in their mouth in the cheek pouch for three hours. According to the dry weight of the remnant expelled at the end of the sucking time, some of the volunteers may have been able to keep the lozenge in their mouth even longer.
As a consequence of this study, it would seem worthwile investigating the value of the amphotericin B lozenges in the preventive treatment as well as in therapy of *Candida* lesions in the oropharynx. The effect in preventive treatment could be expressed in the (reduction of the) number of *Candida*-positive throat cultures in leukopenic patients. On the basis of our findings, presented here, we are inclined to conclude that three to four lozenges per day (one after each meal) could well be sufficient.
Our previous study on amphotericin B concentrations in the saliva after application of it in orabase® promised good results in selective decontamination (16). Lozenges may however be preferable in a number of cases as a result of the stickiness and the rather unpleasant taste of orabase®. Our patients in general prefer the lozenges for long term treatment. Because of the improved palatability of the amphotericin B lozenges over that of amphotericin B in orabase®, a better result could be expected in the suppression or eradication of yeasts from the oropharynx in leukopenic patients.

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CHAPTER VII

THE EFFECT OF AMPHOTERICIN B LOZENGES ON THE PRESENCE AND NUMBER OF CANDIDA CELLS IN THE OROPHARYNX OF NEUTROPENIC LEUKAEMIA PATIENTS *)

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SUMMARY

A retrospective evaluation was made concerning the value of amphotericin B lozenges in selective elimination of yeasts from the oropharynx. Four different groups of severely granulocytopenic patients were studied. All 77 patients received amphotericin B orally as a suspension or as tablets.
Per day four amphotericin B lozenges were added for topical antimycotic decontamination of the oropharynx. This was done in the presence of colonization-resistance decreasing factors such as a nasogastric tube (group I, 19 patients) or mucosal damage (group III, 25 patients) and in patients with four or more consecutive throat swab cultures with yeasts previously (group IV, 11 patients). The 22 patients in group II did not receive lozenges. The addition of lozenges resulted in a decrease in the mean "growth density" of *Candida* cells in the oropharynx. This reduction was significant in group III ($P < 0.01$) and group IV ($P < 0.02$) and became evident during the first week of treatment.
In patients with a nasogastric tube however 51.8% of the throat swab cultures revealed yeasts. Increasing the dose of the lozenges might improve the results in these patients.
Topical treatment of the oropharynx with amphotericin B lozenges is advocated for patients who are susceptible to *Candida* infections.

*) Submitted to: Infection.

INTRODUCTION

Acute leukaemia patients with a low peripheral leukocyte count due to intensive cytostatic therapy are prone to develop infections. The risk of infection is apparently directly related to the degree and the duration of granulocytopenia (1). Most of the infections are caused by representatives of the patient's own oro-intestinal microflora, particularly by aerobic gram-negative rods (2, 3, 4, 5). The number of infections caused by yeasts and fungi is much smaller (2, 5, 6, 7, 8). *Candida* infections usually present as superficial lesions of the skin and the mucous membranes (9, 10, 11, 12), which can penetrate to the depth (6, 7, 13). Disseminated mycotic infections are difficult to treat and have a high mortality rate (5, 6, 7).
Moreover, it has been shown that the digestive tract can also be the port of entry for systemic *Candida* infections (14, 15).
Attempts to reduce the infection risk have been made by total decontamination (16, 17, 18). This is done by means of oral non-absorbable antibiotics in order to sterilize the gut. Potentially pathogenic bacteria are eradicated in this way.
However, also the anaerobic digestive tract microflora, responsible for the colonization resistance (CR) (19), is eliminated by this procedure. The CR of the digestive tract is a physiological barrier which controls the colonization by exogenous potentially pathogenic microorganisms. The importance of the CR, especially as far as yeasts are concerned, has been demonstrated clearly by van der Waaij and Vossen (20). Elimination of *Candida albicans* from the faeces of an otherwise successfully totally decontaminated patient appeared to be only possible after cessation of the CR-decreasing antibiotics and implantation of a human intestinal anaerobic flora.
Therefore, we investigated the value of selective decontamination of the digestive tract as a method of infection prevention in neutropenic patients (21). The purpose of this selective decontamination was eradication of potentially pathogenic bacteria, without affecting the anaerobic CR-responsible microflora. The reduction of the number of infections obtained in the selectively decontaminated group in comparison with an equally sized control group, was most pronounced for those caused by aerobic gram-negative rods (21). The reduction of yeast infections was less impressive.
Prophylaxis against yeasts and fungi is most often performed by oral

administration of nystatin or amphotericin B. In the above mentioned study on selective decontamination of the digestive tract (SDD) in granulocytopenic patients, we have used oral amphotericin B for the suppression or elimination of yeasts inside the alimentary canal.
During the prophylactic use of amphotericin B we found a significant reduction in the number of cultures which were positive for yeasts from both the faeces and the throat swabs. In spite of this reduction *Candida* infections were still found in the selectively decontaminated patients.
The site with most yeast associated lesions in that study was the oropharynx. Attempts were made to reduce the yeast concentration further than was obtained by four times daily oral administration of amphotericin B.
The present study concerns a retrospective mycological evaluation of the effect of topical application of amphotericin B lozenges in the oropharynx. In previous experiments in volunteers it was found that application of amphotericin B lozenges resulted in high concentrations of this drug in the saliva for several hours (22).

PATIENTS AND METHODS

Patients

Seventy-seven patients were studied retrospectively. All patients were treated with intensive chemotherapy, consisting of arabinosyl-cytosine 650 mg/m^2 twice daily for three days, 6-thioguanine 100 mg/m^2 twice daily on the same days, followed by daunorubicin 50 mg/m^2 on three consecutive days. This regime leads to severe granulocytopenia of approximately 2-3 weeks.
Sixty-one courses of chemotherapy were given for AML and 16 for ALL. Both categories of patients were treated with identical chemotherapy.
Four groups of patients were analyzed:

Group I

These patients manifested considerable weight loss and nausea during the first days of chemotherapy and were therefore fed through a nasogastric tube. Antimicrobial drugs for selective decontamination were given through this tube. All the 19 patients in this group were treated with amphotericin B lozenges in addition.

Group II

Patients who were able to maintain their body weight sufficiently by eating the normal hospital food. Drugs for selective decontamination including amphotericin B were given orally. Amphotericin B lozenges were not added (22 patients).

Group III

The 25 patients studied in this group had no nasogastic tube and they received their amphotericin B by mouth together with lozenges because of ulcerative oropharyngeal lesions of the mucous membranes. Lozenges were applied in the treatment of patients with mucosal erosions irrespective whether these lesions were associated with positive yeast cultures or not.

Group IV

These patients received amphotericin B lozenges after four or more subsequent throat swab cultures with yeasts were found, although ulcerative lesions were absent (11 patients).

All patients were selectively decontaminated, whether they were treated with additional lozenges or not. Patients in group I received their SDD-medication through the nasogastric tube. None of the patients in the other groups was fed through a nasogastric tube.

Selective decontamination of the digestive tract

As soon as the peripheral granulocyte count decreased below 500/mm^3 of blood, infection prevention was started. This included oral administration of drugs for elimination of aerobic gram-negative rods. Polymyxin, co-trimoxazole or nalidixic acid were used for this purpose. These drugs were given either alone or in combination. The antifungal part of selective decontamination of the digestive tract (SDD) consisted of 2 grams of amphotericin B orally. This was administered either as a suspension or as tablets. The daily dose was divided into four portions. All drugs for SDD were given orally or via the nasogastric tube in the forced fed patients (group I).

This prophylactic treatment was continued as long as the peripheral granulocyte count was below 500 cells per mm^3 of blood.

Amphotericin B lozenges*)

Topical antimycotic treatment involved amphotericin B lozenges each containing 10 mg of the active substance. The lozenges were administered four times a day and had to be maintained by the patients in their mouth as long as possible.

Mycological monitoring

Throat swabs were taken three times a week from each patient. For the detection of yeasts, the swabs were streaked onto a Yeast Isolation (YI) agar (Merck) and thereafter they were put into a Brain Heart Infusion (BHI) broth (Oxoid) for enrichment. The culture of the swab in BHI broth was subinoculated onto a YI agar after overnight incubation at 37°C. If the broth showed no growth after 18 hours of incubation, the duration of the incubation was extended for another 24 hours before the subinoculation on the YI agar was made. Absence of *Candida* species was concluded when no yeast colonies were seen after maximally 42 hours of incubation of the YI agar at 37°C.

The direct inoculation of the YI agar was done in a semiquantitative way. On the basis of the number of colonies of *Candida* species on the YI agar, the 'growth density" was estimated as +2, +3 or +4. A value of +1 was given when only the broth culture was positive for yeasts and the direct inoculation on the YI agar was negative.

Mean "growth densities" (sum of the "growth densities" divided by the number of cultures) were calculated for each group separately before treatment and thereafter weekly. For statistical reasons this was confined to the first four weeks of treatment only; this was also the average treatment duration.

The effect of the addition of lozenges to oral amphotericin B will be measured by the following parammeters:

- the number of patients with yeasts in their oropharyngeal cultures.
- the number of throat swab cultures with yeasts.
- the mean "growth density" of yeasts in the cultures from the throat swabs.

*) Amphotericin B lozenges were kindly provided by SQUIBB-NEDERLAND.

Statistical analysis

Data concerning the number of patients and the number of cultures were analyzed by the chi-square test (two-sided). The Wilcoxon test was applied to the differences in the mean "growth densities".

RESULTS

The characteristics of the 77 patients on study are given in Table I. At the start of the study the incidence of yeasts in the oropharynx differed not significant between group I, II and III mutually. The patients in group IV were selected on the basis of positive oropharyngeal cultures.

Table I: Characteristics of the patients on study in the different groups

	Group I	Group II	Group III	Group IV
Total number of patients	19	22	25	11
– AML	16	18	19	8
– ALL	3	4	6	3
Lozenges	+	—	+	+
Indication for lozenge treatment:				
– nasogastric tube	+	—	—	—
– ulcerative lesions	—	—	+	—
– >4 positive cultures	—	—	—	—
Number of days on study	516	821	663	243
– mean ± SD	27.2 ± 16.2	37.3 ± 19.2	26.5 ± 9.1	22.4 ± 10.7
– range	8–68	17–93	10–53	8–47
Number of patients with yeasts in the throat swab cultures before they came on study	12	9	15	11

Patients

During the study period no or less than 25% positive throat swab cultures were found in 6, 13 and 19 patients in group I, II and III respectively (Table II).

The results for patients with only a small number of yeasts in their throat swabs (direct inoculation on the YI agar was negative) are given in Table III.

Table II: Culturing results of the throat swabs positive for *Candida* (direct growth or following enrichment in BHI-broth)

	Group I	Group II	Group III	Group IV
Number of cases	19	22	25	11
– with less than 25% of cultures positive	6	13	19	6
Number of cultures	220	337	289	104
– with yeasts	114	102	45	32
– % of positive cultures	51.8	30.3	15.6	30.8
		└P <0.0005┘ └P <0.0005 ┘		

Tabel III: Culturing results of the throat swabs positive for *Candida* when directly streaked on YI agar.

	Group I	Group II	Group III	Group IV
Number of patients	19	22	25	11
– with less than 25% of cultures positive	11	15	23	6
		└ P <0.05 ┘		
Number of cultures	220	337	289	104
– with yeasts	60	75	27	19
– % of positive cultures	27.3	22.3	9.3	18.3
		└ P <0.0005 ┘		

One week after the start of the additional lozenges 6 out of the 11 patients in group IV had got negative throat swab cultures and maintained so during the rest of their period on study.

Cultures

The lowest incidence of positive throat swab cultures was found in group III with 15.6%. This figure was significantly lower than the 30.3% in group II (Table II).

Patients with a nasogastric tube however, had significantly more throat swab cultures with yeasts than patients in the other groups.

Table III gives the results when only those cultures are included which were positive after direct inoculation of the YI-agar. In this way the percentage of positive cultures decreased to 18.3% in group IV.

Estimated mean "growth density"

The mean "growth density" of 1.7 in group IV before the addition of the lozenges was significantly higher than in group II ($P < 0.01$) (Figure 1). In all groups there was a decrease in the mean "growth density" during the first week of treatment, which was significant for group III ($P < 0.01$) and group IV ($P < 0.02$). During the following weeks of treatment this reduction of the mean "growth density" progressed in group III and IV. In group I and II however, the "growth density" remained rather constant after the initial reduction obtained during the first week of treatment (Figure 1).

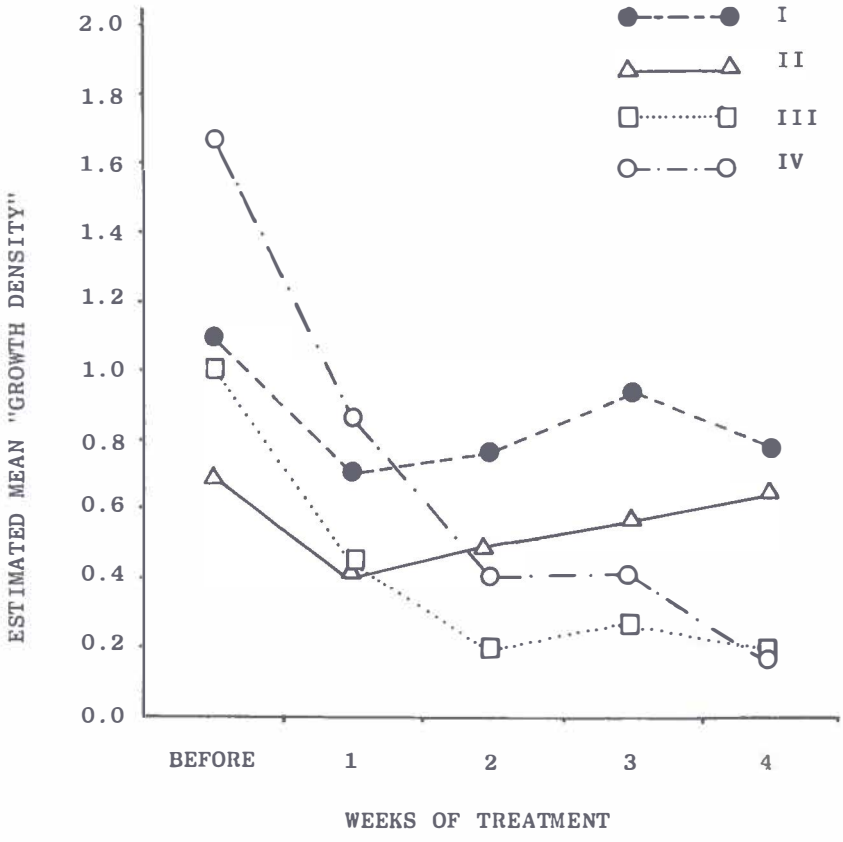

Figure 1: Estimated mean "growth density" of the throat swab cultures in the different groups before treatment and during four weeks thereafter.

DISCUSSION

The results of this retrospective evaluation indicate that selective decontamination of the oropharynx regarding yeasts could be improved when amphotericin B lozenges were used in addition to the original prophylactic treatment including amoung else 2 grams of amphotericin B per day orally. In a previous study we have measured salivary concentrations of amphotericin B after application of this drug in orabase® (23). Similar experiments were performed with amphotericin B lozenges (22). Both forms of application of amphotericin B resulted in high antimycotic concentrations in the saliva, which could be maintained for several hours. Because of the better palatability of the lozenges, the latter form was considered to be more suitable for long term use. Ewing treated 81 patients with this drug for oral thrush (24). Mycological cure was obtained in 73% of the patients, after one week of treatment with four lozenges daily.

We have rejected clinical evaluation because it has a very subjective component, particularly in leukaemic patients during remission induction therapy. This therapy in itself often causes mucosal damage. The percentage of positive throat swab cultures found during lozenge treatment appeared a more objective analysis. This figure was lowest (15.6%) in the group in which oral lesions formed the indication for lozenge treatment, group III. When throat swab cultures with a low "growth density" (those which were given a value of +1) were considered negative, this figure further decreased to 9.3%. Moreover, the number of patients with no or only a low yeast concentration in the oropharynx was significantly lower in group III as compared with the control group, i.e. patients who were not additionally treated with lozenges.

The therapeutic effect of the lozenges was also obvious in the patients treated because they had repeatingly positive throat swab cultures. Previous administration of oral amphotericin B alone had failed to produce negative throat swab cultures in them. At least four subsequent positive cultures had been found in these patients previous to lozenge treatment. This means that amphotericin B had been administered for minimally ten days before the addition of the lozenges to the SDD-regimen. In six out of the eleven patients in this group, the oropharyngeal cultures became negative for yeasts within one week after the onset of the topical antimycotic treatment. This situation could be maintained during the rest of the observation period of these patients.

Prophylaxis by amphotericin B lozenges is however, not optimal under all circumstances. In patients who had a nasogastric tube for the administration of food, the drugs for selective decontamination were also given via the tube. In this way the oral cavity was not exposed to the amphotericin B. The number of throat swab cultures with yeasts remained quite high in these patients (51.8%). Some patients who at admission had appeared negative for *Candida* species in their throat swab cultures, became positive during lozenge treatment. Perhaps this could be ascribed to both the presence of the nasogastric tube, a foreign body, which may have enhanced *Candida* proliferation, or, what seems more plausible, to the clinical condition of these patients. They were forced fed through a nasogastric tube because they lost body weight. Abnormal colonization of the oropharynx by for example gram-negative bacteria is a common feature in such patients (25).

The nasogastric tube may also have caused a reduction of the CR of the oropharyngeal area, because of reduced mechanical cleaning. The absence of oral food intake may have reduced the saliva production, for sure they did chew less and had less vigorous movements of their tongue.

The actual effect of the lozenge treatment in this category of essentially catabolic patients can however not be concluded from this study. We did not have a control group of patients, i.e. patients who were forced fed through a nasogastric tube but were not treated with lozenges. On the basis of the good results obtained in other patients who were supplied with lozenges because of mucosal erosions or an alarmingly number of positive throat swab cultures, it is to be expected that our group I patients would have done worse without lozenge treatment. A more frequent application of the lozenges, for example six or eight per day, may have better results in patients with a nasogastric tube.

We tend to conclude on the basis of our findings that in patients prone to get *Candida* infections, topical treatment of the oropharynx area with amphotericin B lozenges is to be strongly recommended. It is a treatment with a good acceptabillity. In addition it has a good mycologically documented effect.

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CHAPTER VIII

SUMMARY

This thesis consists of a series of studies dealing with the approach of life-threatening infections which patients may experience during periods of severe granulocytopenia.

In the introduction it is pointed out that most of these infections are caused by aerobic gram-negative bacteria and yeasts. These microorganisms originate from the patient's own oro-intestinal microflora. Since these infections are often difficult to treat and have a high mortality rate, attempts have been made to prevent them by total decontamination with or without reverse isolation. In total decontamination all microorganisms including the anaerobes are eliminated as far as possible.

On the basis of quantitative data, obtained in animal experiments, anaerobic bacteria are believed to confer resistance to colonization of the host gut by exogenous environmental microorganisms which are potentially pathogenic. This natural barrier has been called Colonization Resistance (CR).

Therefore we tried to develop a new approach to infection prevention, based on maintaining the CR in the clinical situation and on eliminating only aerobic gram-negative rods and yeasts. This procedure is referred to as Selective Decontamination of the Digestive tract (SDD). Aerobic gram-negative rods can be eliminated by oral administration of the non-absorbable polymyxin or by the absorbable drugs co-trimoxazole and nalidixic acid. For the eradication of yeasts and fungi amphotericin B and nystatin are most frequently used.

Chapter II consists of a preliminary report of a prospectively controlled randomized study in which adequate doses of these drugs were administered to patients during periods of severe granulocytopenia. A more extensive report of this study is given in the thesis of Sleijfer (Groningen, 1981). The incidence of infections was significantly reduced in the selectively decontaminated patients as compared with a not-prophylactically treated control group.

The clinical effectiveness of selective decontamination of the digestive tract was found to correlate well with the almost complete reduction of the aerobic potentially pathogenic bacteria in these patients (Chapter III).

When antimicrobial drugs are applied orally, questions always arise about

the possible emergence of resistant microorganisms. This topic is considered in Chapter IV. During SDD the development of resistance was not seen.

The reduction of the frequency of fungal infections during SDD was less impressive than that of bacterial infections. Most of the yeast associated lesions were found in the oropharynx. This is in concordance with the bacteriological observation that selective decontamination of the oropharynx was more difficult than that of the colon, especially as far as yeasts were concerned. Therefore we searched for methods to improve selective elimination of yeasts from the oropharynx.

By mixing amphotericin B with orabase® and applying this paste to the mucous membranes of the mouth in healthy volunteers, high salivary concentrations of this drug were obtained and could be maintained for several hours (Chapter V).

In Chapter VI similar experiments with amphotericin B lozenges are described. As long as the volunteers kept the lozenges in their mouths, high antimycotic concentrations were measured in the saliva.

In chapter VII the effect of the application of amphotericin B lozenges to reduce the yeast concentration in the oropharynx of leukaemic patients was investigated. Topical treatment of the mouth in addition to the standard regime of SDD, appeared to improve the elimination of yeasts from this region. However, in the presence of a nasogastric tube, through which the SDD drugs were administered, local application of the lozenges was less sufficient.

In conclusion, it may be said that oral administration of antimicrobial drugs in sufficient dosages without destroying the CR, proved effective in the clinical situation as investigated by us.

This new principle, developed in an animal model, therefore provides an important guideline in searching for measures to prevent acquired infections.

Practical advantages of the presented approach are that patients can be treated in normal hospital rooms of 4 or 6 beds without isolation measures and that they can reveive a normal hospital diet. This method can therefore be used in hospitals without special equipment such as laminar air flow units for reverse isolation of the patients. It should however be stressed that intensive bacteriological surveillance remains necessary in order to guarantee that the oral administration of the drugs for SDD really results in elimination of aerobic gram-negative rods and yeasts from the digestive tract.

ENKELE HOOFDLIJNEN UIT DIT PROEFSCHRIFT

Witte bloedlichaampjes vormen een belangrijk onderdeel van het normale verdedigingsmechanisme tegen de ons altijd en overal omringende micro-organismen. Bacteriën en gisten behoren tot deze categorie en de meesten zijn potentieel pathogeen. Dat wil zeggen dat ze pas infecties veroorzaken als de weerstand van de gastheer afneemt.
Huid en slijmvliezen zijn de eerste barrières die bacteriën tegenkomen bij hun poging tot invasie. Bij een venapunctie echter – het aanprikken van een ader – wordt de continuïteit van de huid doorbroken.
Slijmvliezen kunnen worden aangetast door het gebruik van cytostatica, stoffen die delende cellen remmen in hun groei. De effecten zijn vooral merkbaar in snel delende weefsels zoals slijmvliezen en beenmerg. Cytostatica worden gebruikt bij de behandeling van kanker. Als bijwerking van deze therapie kan ook de groei van andere dan kwaadaardige sneldelende weefsels – zoals beenmerg en slijmvlies – worden geremd. Aantasting van de functies van deze organen heeft een verminderde weerstand tegen infecties tot gevolg.
Bij patiënten met bloedkanker (leukemie) is het productieproces in het beenmerg (bloedvormend orgaan) ontspoord: in plaats van de normale cellen (witte bloedlichaampjes, rode bloedlichaampjes en bloedplaatjes) worden kankercellen aangemaakt. Deze patiënten worden behandeld met cytostatica om de kankercellen uit te roeien. Meestal echter verdwijnt ook het laatste restje normale witte bloedcellen. Gewoonlijk duurt deze situatie waarin de patient geen witte bloedcellen heeft (granulopenie) 2 à 3 weken. In de granulopenische periode heeft de patiënt – als gevolg van de verminderde weerstand – bijna 100% kans op het krijgen van een infectie. Het is gebleken dat de meeste van deze infecties worden veroorzaakt door de zogenaamde facultatief aerobe (die zowel met als zonder zuurstof kunnen leven) bacteriën uit het maagdarmkanaal. Ze worden potentieel pathogeen genoemd en vormen ongeveer 1/100 deel van de in de darm aanwezige bacteriën. De overgrote meerderheid van de bacteriën in de darm zijn obligaat anaeroob, dat wil zeggen dat ze niet tegen zuurstof kunnen. Zij veroorzaken zelden infecties bij leukemiepatiënten. Integendeel, zij vervullen juist een nuttige functie, die Kolonisatie Resistentie (KR) wordt genoemd. Dit is het mechanisme dat het potentieel pathogene bacteriën moeilijk maakt zich te vestigen in het maagdarmkanaal.
Dit proefschrift behandelt een op het in stand houden van de KR gebaseer-

de methode voor ontsmetting (decontaminatie) van het spijsverteringskanaal om bij patiënten met verminderde weerstand infecties te voorkomen: alleen de potentieel pathogenen worden verwijderd uit de bron (het maagdarmkanaal) en de anaeroben, die verantwoordelijk zijn voor de KR, worden gespaard. Deze procedure wordt selectieve darmdecontaminatie (SDD) genoemd.
Voor wat betreft de bacteriën kan een keuze worden gemaakt uit de volgende antibiotica: nalidixinezuur, co-trimoxazole en polymyxine. Gisten kunnen worden verwijderd met behulp van amphotericine B of nystatine.
In Hoofdstuk II wordt het effect van deze profylactische behandeling, dat is de behandeling om te voorkomen dat de patient infecties krijgt, besproken. Selectief gedecontamineerde patienten bleken veel minder infecties te krijgen dan vergelijkbare patiënten zonder SDD.
Dat de afname van het aantal infecties inderdaad op rekening komt van het opruimen van de bacteriën in de bron (het maagdarmkanaal) blijkt uit Hoofdstuk III. Tijdens het onderzoek werd namelijk 3 maal per week van elke patient een keeluitstrijk (het begin van het maagdarmkanaal) en ontlasting (weerspiegelt de bacteriële situatie in de dikke darm) bacteriologisch onderzocht. Het aantal kweken met potentieel pathogenen bacteriën was in de SDD- groep significant lager dan in de controle groep. Dit verschil was echter duidelijker in de kweken van de ontlasting dan in die van de keeluitstrijken. Dit is niet zo verwonderlijk als we bedenken dat de antibiotica die we voor SDD gebruiken vrijwel direkt na inname worden doorgeslikt en zo nauwelijks in contact komen met de in de mond aanwezige micro-orgamismen. In de dikke darm komt wel voldoende antibioticum om gevoelige bacteriën te doden.
Het gedurende langere tijd profylactisch geven van antibiotica kan ongevoeligheid (resistentie) van bacteriën in de hand werken. Bij de patiënten die bij dit onderzoek betrokken waren trad geen toename van het aantal resistente stammen op (Hoofdstuk IV).
Bacteriologisch onderzoek leerde dat bacteriën moeilijker uit de mondkeelholte waren te verwijderen dan uit de darm. In het bovenste gedeelte van het spijsverteringskanaal zijn het vooral de gisten die veel voorkomen en ook infecties kunnen veroorzaken. Vandaar dat gezocht werd naar methoden om gedurende langere tijd een zodanige concentratie amphotericine B in het speeksel te handhaven, dat gisten worden gedood. Dit kan worden gedaan door dit middel te mengen met een pasta die goed blijft

plakken aan het mondslijmvlies. Deze pasta heet: orabase®. Van hieruit wordt de amphotericine B afgegeven aan het speeksel.
Het bleek dat gedurende 3 à 4 uur de amphotericine B concentratie in het speeksel voldoende hoog was om gevoelige gisten te doden. Wanneer deze pasta 's avonds voor het slapen gaan werd aangebracht op het mondslijmvlies, bevatte het speeksel de volgende ochtend nog veel amphotericine B (Hoofdstuk V).
Een andere manier om hoge amphotericine B speeksel concentraties te bewerkstelligen is het gebruik van zuigtabletten. Vrijwilligers bleken in staat te zijn de zuigtabletjes ongeveer 3 uur in de mond te houden. Gedurende deze periode en ook nog een half uur na het doorslikken van de laatste rest van de zuigtablet, was de gistdodende concentratie in het speeksel voldoende hoog, dwz. ver boven de concentratie die minstens nodig is om gisten in hun groei te remmen (Hoofdstuk VI).
Beide manieren om gedurende langere tijd een hoge amphotericine B concentratie in het speeksel te bewerkstelligen, werden onderzocht bij vrijwilligers. Over het algemeen werden de zuigtabletten als prettiger ervaren dan de orabase®. Vandaar dat de zuigtabletten werden gebruikt voor de behandeling van patiënten. Patiënten die naast de middelen voor SDD ook nog amphotericine B zuigtabletten kregen, hadden minder keeluitstrijken met gist, dan patiënten die dat niet kregen. Een aantal patiënten die tijdens de leukemie behandeling sterk afvielen werden echter gevoed via een maagsonde (slang die door de neus wordt ingebracht en in de maag uitkomt). Ook de medicamenten voor SDD werden via deze sonde toegediend. Op deze manier kwam er bij deze ernstig zieke mensen geen amphotercine B in de mondholte terecht. Ter compensatie van dit nadelig effect werden amphotericine B zuigtabletten toegevoegd. Het effect hiervan was echter onvoldoende, aangezien deze patiënten meer gist in hun keeluitstrijken bleken te hebben dan patiënten zonder maagsonde die geen zuigtabletten kregen (Hoofdstuk VII).
De in dit proefschrift onderzochte methode om infecties te voorkomen bij patiënten die daar erg gevoelig voor zijn, is gebaseerd op het intact laten van de KR. De waarde hiervan voor de patient is duidelijk gebleken uit de significante daling van het aantal infecties bij selectief gedecontamineerde patienten ten opzichte van die bij een controle groep zonder profylactische behandeling. Wil men echter selectieve darmdecontaminatie met succes toepassen dan is een goede samenwerking tussen allen die bij de behandeling van dergelijke patiënten zijn betrokken, inclusief de bacterioloog, een eerste vereiste.